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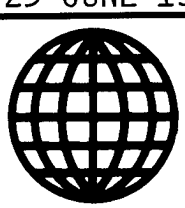

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EXPERIMENTAL AND GENERAL THEORETICAL RESEARCH

UDC: 629.78:[61.001.8]"1985"

RESULTS OF MEDICAL RESEARCH CONDUCTED IN 1985 DURING LONG-TERM SPACEFLIGHTS

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 22, No 1, Jan-Feb 88 (manuscript received 22 Jan 87) pp 4-7

[Article by A. D. Yegorov, O. D. Anashkin, O. G. Itsekhovskiy, I. V. Alferova, Z. A. Golubchikova, V. R. Lyamin, A. P. Polyakova, V. F. Turchaninova, V. A. Talavrinov, and V. D. Turbasov]

[English abstract from source] This paper presents medical results obtained during the fourth expedition of five cosmonauts onboard orbital complexes Salyut-T—Soyuz-T-13 and Salyut-7—Soyuz-T-14. The cardiovascular system was examined using 36 resting and provocative tests. They were performed by means of electrocardiography, tetrapolar rheography, arteriovenous pulsography and tachooscillography. In addition, body mass and leg volume were measured. The above parameters showed typical variations as well as individual changes related to the preflight circulation level and environmental effects. The use of modified regimens of provocative tests demonstrated their applicability to the assessment of cardiovascular function in space flight.

[Text] In 1985, medical research was continued in the USSR during long-term flights aboard Salyut-7—Soyuz-T-13 and Salyut-7—Soyuz-T-14 orbital complexes. During these missions there was partial replacement of the crew in orbit. The crew members were distributed as follows, according to duration of their stay in space: 169 days—1 cosmonaut, 113 days—1, 65 days —2 and 10 days—1.

At the first stage of the mission, when the station was being overhauled and restored, the basic ambient parameters, temperature and humidity deviated from nominal values, which required performance of special preventive, sanitary-hygienic and medical measures. After completion of repair and overhaul work on the station, the habitat provided for the appropriate comfort level.

Cosmonauts adhered to a work and rest schedule, that enabled them to retain the correct sleep-waking cycle and required efficiency. They slept adequately and were refreshed after sleeping an average of 7-8 h. As a rule, the cosmonauts exercised daily using exercise equipment.

Throughout the missions, the cosmonauts felt well according to their own rating. Their psychoneurological status remained in the normal range.

Anthropometric studies revealed changes in weight and lower leg volume. The noted weight loss is inherent in spaceflights. At the same time, the crew members lost more weight during the first mission than in previous flights. Maximum weight loss was recorded in the 1st month of the flight (4.4 and 6.3 kg). This can apparently be attributed to the greater expenditure of energy during the first weeks of the mission. The tendency toward restoration of weight (Figure 1) confirms this assumption indirectly. After the initial weight loss of 2.2-2.3 kg by the crew of the second expedition, their weight did not change appreciably to the end of the flight (Figure 2).

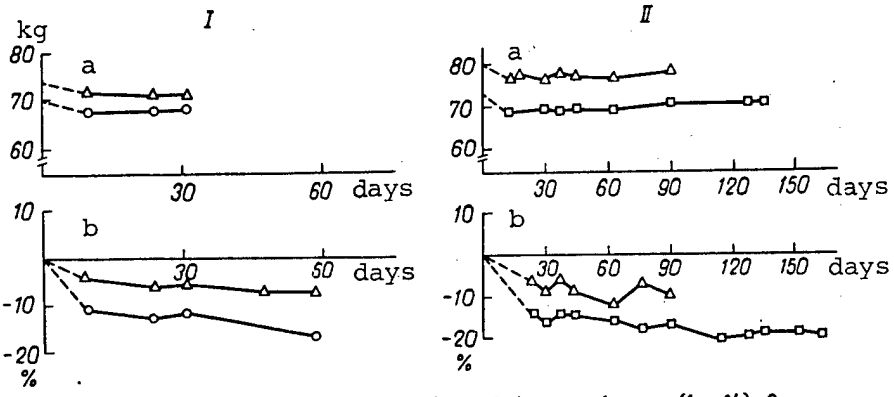


Figure 1. Dynamics of weight (a; in kg) and leg volume (b; %) for crews of first (I) and second (II) EO-4 inflight

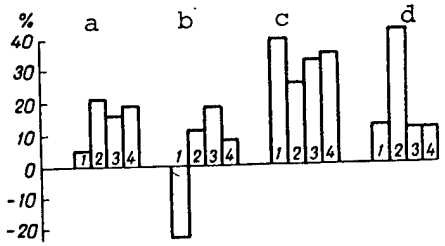


Figure 2.
Inflight changes in hemodynamic parameters of EO-4 crew members at rest ($\pm\Delta\%$, in relation to mean preflight values)
a) heart rate
b) cardiac output
c) arterial pulse pressure
d) hemodynamic stroke
Here and in Figures 3 and 4:
1-4 refer to crew members

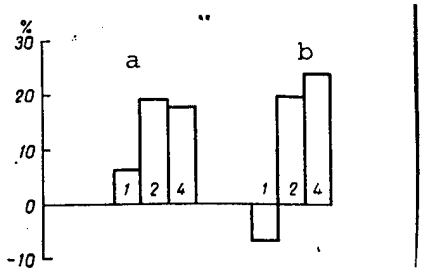


Figure 3.
Changes in heart rate (a) and cardiac output (b) during flight at rarefaction of —45 mm Hg in EO-4 crew members ($\pm\Delta\%$, in relation to mean preflight values during LBNP

We conducted 36 studies of cardiovascular function, which included examination of bioelectric activity of the heart, its mechanical activity, circulatory volumes and parameters of systemic arterial pressure; filling and tonus of regional vessels (of the head, arm and leg), pressure in the jugular vein. As in previous investigations, electrocardiography, tetrapolar rheography, venous-arterial pulsography and tachooscillography were used.

Analysis was made of data obtained at rest and during functional tests: with use of stepped lower body negative pressure (LBNP) (—25, —35, and —45 mm Hg for 1, 3 and 3 min, respectively) and with two-stage graded exercise (TGE) on a cycle ergometer (125 W for 5 min, 1-min pause, 175 W for 3 min).

At rest, parameters characterizing cardiovascular system function did not exceed the range of the physiological norm in any of the cosmonauts. During long-term missions it was established that there was consistent decline of stroke volume, minimum arterial pressure, pulsed filling of crural vessels (by 18-40%) and tonus of small (mainly precapillary) vessels in the region of the forearm and pools of the internal carotid arteries (on the right and left) in 3 cosmonauts. In addition, there was a tendency, to some degree or other, toward increase in heart rate, systolic components of arterial pressure, arterial pulse pressure and hemodynamic stroke (difference between lateral and end systolic pressure (see Figure 2).

The deviations from preflight levels of a number of hemodynamic parameters were dissimilar in the first crew who were exposed to identical ambient conditions and performed the same operator work. For example, one of the cosmonauts presented decline of minimal and mean dynamic pressure, appreciable decline of stroke volume (by 29%), cardiac output (by 21%) and increase (by 20%) in actual specific peripheral resistance which, however, was below nominal values during the flight (the ratio constituted 87.7%). These changes occurred in the presence of relatively high (preflight) circulatory volumes and peripheral resistance.

In another cosmonaut on the same mission, all parameters of arterial pressure rose. Circulatory volumes, which were lowest preflight for this group of cosmonauts, changed over a narrow range in weightlessness (stroke volume had a tendency toward decline, while cardiac output had a tendency toward increase). Specific peripheral resistance, which differed insignificantly from preflight values, exceeded (by 11%) the nominal value (the preflight ratio between them was 98.7%).

Pulsed filling of vessels of the brain increased in both cosmonauts of the firsst basic mission (more so on the left), and this led to development of interhemispheric asymmetry with relatively more delivery of blood on the left. Venous pressure in the jugular vein (12.3—32.5 mm Hg) was 2—3 times higher inflight than preflight (6.7—8.4 mm Hg).

LBNP, which simulated redistribution of blood in orthostatic position, was generally associated with consistent changes in the circulatory system due to deposition of blood in the lower half of the body. In addition, there were typical distinctions that were manifested by a faster heart rate, less marked reaction of stroke volume and virtually no changes in peripheral resistance under the effect of rarefaction (before the flight this parameter always increased; Figure 3).

In addition, each cosmonaut presented individual deviations. In one case they were characterized by marked increase in heart rate to 84—92/min (preflight this parameter did not exceed 71—76/min); low minimal arterial pressure, stroke volume and cardiac output; decline in ratio of actual specific peripheral resistance to nominal to 84.6% (preflight 91.9%). Use of LBNP normalized the tonus of small cerebral vessels. Dicrotic index on the rheoencephalogram rose to preflight values during the test, and it was indicative of normotonic precapillary vessels in the pool of the internal carotid artery.

In another cosmonaut, heart rate also increased under the influence of rarefaction, but with concurrent increase in stroke volume and particularly cardiac output, as compared to preflight tests (under analogous conditions). Specific peripheral resistance during LBNP showed virtually no change while its ratio to nominal value (106.9—107.4%) exceeded preflight values. Tonus of small cerebral vessels increased by 43.6-68.0% and was essentially in the range of values inherent in hypertensive arterioles.

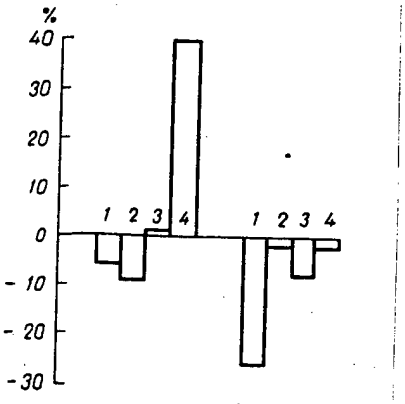


Figure 4.
Changes in parameters of work capacity (a) and cardiac output (b) during flight with graded physical exercise on cycle ergometer (125-175 W for 5 min) in crew of EO-4 ($\pm\Delta$%, as compared to mean preflight values)

With rarefaction of —45 mm Hg, stroke volume decreased more (by 32—40%) than at —35 mm Hg (by 7—31%). At the same time, the changes in most of the other tested parameters were less marked at —45 mm Hg than at —35 mm Hg. This can be interpreted as a manifestation of adequate reserve compensatory capabilities of the cardiovascular system.

In most of the tests with TGE performed in flight, the changes in parameters due to physical exercise in weightlessness did not exceed the range of fluctuations in which tolerance could be rated as being good.

Even when conditioning diminished (8—20% decrease in work capacity), on the whole it remained rather high, in spite of faster heart rate than before the flight (Figure 4).

After inflight exercise, cardiac output was at the level inherent in weightlessness in two cosmonauts due to the predominant influence of chronotropic function of the heart with virtually no response referable to stroke volume or its decline. This was manifested the most distinctly after the second stage of exercise. In the other two cosmonauts, during the inflight test cardiac output level was attributable, as it was before the flight, to increase in heart rate and stroke volume. However, with increase in intensity of exercise there was some decrease in role of stroke volume (from 17.6% to 9—13%).

Electrocardiography failed to demonstrate pathological changes in bioelectrical activity of the myocardium both at rest and during functional tests. At the same time, all of the cosmonauts presented a decline in amplitude of T waves in most leads without appreciable changes in their shape, which is typical of long-term flights. Starting with the first month of the flight, two cosmonauts presented extension of electrical systole as compared to nominal values. The dynamics of electrocardiographic parameters reflected changes in myocardial metabolism, in the genesis of which neurohumoral changes in central and peripheral circulation and fluid-electrolyte metabolism could be involved.

In summary, it should be noted that the fluctuations of tested physiological parameters at all stages of flight did not exceed the permissible range, they were adequate

UDC: 612.13-06:612.766.1

HUMAN HEMODYNAMICS DURING WATER IMMERSION AS RELATED TO POSITION DURING SUBMERSION

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 22, No 1, Jan–Feb 88 (manuscript received 4 Mar 87) pp 7–10

[Article by A. M. Genin, A. Yu. Modin and V. S. Shashkov]

[English abstract from source] Central and peripheral hemodynamics was investigated in 16 essentially healthy volunteers who performed a routine tilt test or a tilt test in water immersion. Unlike tilt tests carried out before water immersion, the supine to upright transfer in water did not change cardiac rhythm, cardiac output, leg blood flow or other circulation parameters. The fact that there are no posture-related circulation changes in water immersion suggests that the horizontal and upright positions in water can be viewed as hemodynamically similar.

[Text] It is known that in the course of ordinary activity posture-related fluctuations of a number of parameters occur numerous times daily in the circulatory system. These fluctuations are attributable to differences in hydrostatic load in the direction of the longitudinal axis of the body's great vessels [2]. Posture-determined hemodynamic effects of gravity are not homogeneous, and they reflect both passive mechanical processes of redistribution of circulating blood and active compensatory reactions aimed at maintaining circulatory homeostasis.

If the overall vector of forces acting upon the body is close to zero, there is also disappearance of physical conditions for posture-determined hemodynamic differences. Such a situation is encountered during spaceflights, and it is referred to as weightlessness; on the ground, it is partially simulated by water immersion [3]. In this case, compensation for hydrostatic pressure of blood and tissue fluids is effected by analogous hydrostatic pressure of water, thereby eliminating postural hemodynamic differences. However, total compensation for hydrostatic pressure of blood can be achieved only if its specific gravity is the same as that of the immersion fluid, as well as if this fluid fills all cavities of the human body that contain air. While the first condition can be met rather precisely, the second one is virtually impossible to meet, mainly because of the lungs that are filled with air and are of considerable size in man. As a result, hydrostatic pressure in the lungs remains uncompensated, and it depends on geometric dimensions of the lungs and axis of the body's position in relation to the gravity vector, which could affect central and peripheral circulation.

Our objective here was to determine the distinctions of human circulation as related to horizontal and vertical orientation of the body's longitudinal axis during water immersion.

Methods

These studies were conducted on 13 essentially healthy male volunteer subjects 22—44 years old. Each subject was submitted to water immersion for 2-3 h (he submerged up to the neck) in a large tank 3.8×2.0×2.0 m in size. Air temperature was in the range of 22—25°C during the tests. Nine of the 13 subjects were immersed at a water temperature of 30.5—32.5°C, which conformed to their subjective temperature comfort level, and 4 were immersed in a medium at 35°C temperature, i.e., somewhat above the temperature-neutral level. Methodologically, the studies enabled us to record physiological parameters during immersion.

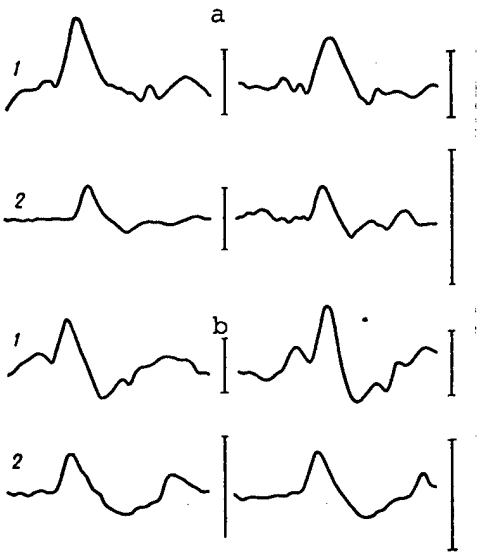
Central and peripheral hemodynamics of the subjects were studied before submersion in water and in the 2d—3d h of immersion. We recorded parameters in recumbent and upright positions, for which purposes we performed the standard orthostatic test before immersion; it involved passive change from horizontal to close to vertical (+70°) position, whereas during immersion this change in position was performed actively by the subjects themselves. Stroke (SI) and cardiac (CI) indexes, blood expulsion period (E), as well as duration of cardiac cycle (C) were determined by tetrapolar thoracic rheography in the modification of A. M. Genin et al. [1] using an RPG2-02 domestic rheoplethysmograph. Longitudinal tetrapolar rheovasography was used to determined pulsed delivery of blood and minute blood flow in the region of the left leg (MFL), as well as pulse wave propagation time (PWPT). Rheographic curves were recorded on a 6NEK-4 instrument, successively for calculation of volumetric features of central and regional blood flow and synchronously for calculation of PWPT. A sequence of rheographic complexes corresponding to one respiratory cycle was submitted to processing. Reliability of differences found was assessed using nonparametric criteria.

Results and Discussion

Ordinary clino-orthostatic testing revealed shortening of C and E, decline of PWPT, SI, CI and MFL, as well as change in informative correlation between the last two parameters.There was reliable decrease by a mean of 20% ($p<0.05$) in ratio of local blood flow in the leg to total circulation volume, the dynamics of which can be viewed as one of the parameters of regional hydraulic vascular resistance. These changes in circulatory parameters were observed in each case, and their intensity corresponded to the known physiological norms (Table 1).

During water immersion there was appreciable change in some hemodynamic parameters. This was manifested primarily by increase in SI and CI, which exceeded levels recorded at clinostatic rest before immersion by a mean of 20% ($p<0.05$) and 28% ($p<0.01$), respectively. Testing revealed that CI constituted 4.112 ± 0.188 $l/\text{min}\cdot\text{m}^2$ (it increased by 8-58%) in a neutral-temperature environment and 4.289 ± 0.171 $l/\text{min}\cdot\text{m}^2$ at water temperature of 35°C (20-70% increase).

Unlike standard orthostatic test conditions, variation of position during immersion was not associated with appreciable changes in graphic parameters of recorded rheograms (see Figure), or in time and volume characteristics of circulation. The absence of



Differential rheograms of thoracic region and leg during ordinary orthostatic test (a) and with immersion (b)

Left—body in horizontal position; right —vertical

1) transthoracic rheogram
2) rheovasogram of leg

Vertical marks refer to calibration signal of 1 Ω/s. Paper feed rate 100 mm/s

Table 1.
Baseline hemodynamic parameters during orthostatic test (from data for 13 cases)

Parameter	Horizontal position	Vertical position
C, s	0,881±0,022	0,688±0,030*
E, s	0,291±0,006	0,227±0,007*
PWPT, s	0,160±0,004	0,128±0,002**
SI, mℓ/m^2	47,5±3,6	29,8±2,4*
CI, ℓ/min•m^2	3,175±0,198	2,485±0,185*
MFL, mℓ/min	157,1±10,4	101,9±6,4**

Note: Parameter E determined with consideration of protodiastolic interval.
*$p<0.01$ **$p<0.05$

consistent changes in central and peripheral hemodynamics with change in position of longitudinal body axis in the water was observed both at comfortable temperature for the subjects and at water temperature exceeding the comfort level (Table 2). There were changes in different directions in values of C, E, PWPT, as well as SI, CI, MFL and ratio of MFL to cardiac output when changing from horizontal position in water to vertical submersion, and in most cases the changes consisted of insignificant deviations within the range of the permissible margin of error of measurements. We also failed to detect differences between horizontal and vertical body position during immersion with regard to thoracic fraction of circulating blood volume. In the former case, impedance constituted 18.75±1.38 Ω and in the latter, 18.70±1.44 Ω, whereas during the preceding orthostatic test we observed not only higher absolute values for this parameter in baseline recumbent position (23.82±1.93 Ω) but a consistent tendency toward their increase due to change to static orthostatic position (25.64±2.20 Ω).

The findings warrant consideration of the horizontal and vertical variants of immersed body position as hemodynamically similar states. Since postural factors and, in particular, the orthostatic test under ordinary conditions are associated with changes in heart rate and vascular hydraulic resistance, which reflect the process of compensatory change in circulation in response to redistribution of blood, absence of such changes during immersion is an indirect indication of the fact that a change in position of the body under such conditions does not lead to further changes in passive mechanical redistribution of blood elicited by the effect of immersion. In contrast to the standard orthostatic test, the analogous test during immersion is not a factor that has an unequivocal effect on blood redistribution. The effect of gravity, which is always associated with change to upright position due to shifting of blood in a caudal direction, is not present during water immersion due to compensation of hydrostatic blood

Table 2.

Dynamics of time and volume characteristics of circulation with change to upright position while immersed

Parameter	Group	Change in relation to floating position, %	
		mean	fluctuat.
C, s	A	—2,2	—17—+14
	B	+3,3	—3—+7
E, s	A	—0,6	—6—+5
	B	—1,7	—3—0
PWPT, s	A	—	—
	B	+0,3	—3—+5
SI, ml/m^2	A	+1,6	—9—+10
	B	—1,7	—12—+12
CI, l/min·m^2	A	+0,3	—10—+11
	B	—5,0	—17—+6
MFL, ml/min	A	—	—
	B	+0,4	—9—+13

A) neutral immersion temperature (9 cases)

B) 35°C water (4 cases) (9 cases)

pressure by pressure of the immersion fluid. On the other hand, prevalence of extrathoracic pressure over intrapulmonary pressure during immersion is a factor that causes noticeable "centralization" of circulation, and if we consider the differences between longitudinal and anteroposterior dimensions of the lungs, we can expect that upright body position will be associated with more marked "centralization" of circulating blood. Nevertheless, we failed to detect noticeable changes in hemodynamics or redistribution of blood with change in submerged body position. Evidently, the differences in longitudinal and anteroposterior geometric dimensions of the lungs are not significant enough to cause noticeable circulatory changes during immersion in water.

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UDC: 616.1-009.86-06:612.014.477-064]-07;616.151.5

HEMOSTASIS PARAMETERS OF INDIVIDUALS WITH NEUROCIRCULATORY DYSTONIA SUBMITTED TO 'DRY' IMMERSION

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 22, No 1, Jan–Feb 88 (manuscript received 8 May 87) pp 10–13

[Article by L. L. Kirichenko, V. P. Masenko, A. B. Raskurazhev and A. G. Yevdokimova]

[English abstract from source] Twelve volunteers, aged 45-55 years, with hypertension type neurocirculatory dystonia were exposed to 7-day "dry" immersion. Plasma, platelet and vessel hemostasis was investigated., "Dry" immersion was found to stimulate hypercoagulatory changes in the above hemostasis systems. It was also shown that the test subjects developed a slow process of readaptation.

[Text] According to data in the literature, the process of human adaptation to weightlessness occurs with considerable loss of plasma, increase in hematocrit and viscosity of blood [1, 4, 9], which could cause impairment of its rheological properties and onset of ischemia of various organs and tissues even to the extent of thromboembolic complications [2, 3].

This study was conducted in order to investigate plasma, thrombocyte and, in part, vascular hemostasis in individuals with neurocirculatory dystonia of the hypertensive type during 7-day exposure to "dry" immersion.

Methods

The model of "dry" immersion was used to simulate weightlessness. The studies were conducted on 12 male subjects 45-55 years of age with neurocirculatory dystonia of the hypertensive type (WHO classification). Hemostasis parameters were measured before immersion, on the 3d and 7th days of immersion and 5 days later. Fasting blood was drawn in the morning, with the subjects in supine position. Thromboelastography (TEG) followed by calculation of 11 parameters was performed using a domestic GKGM-04 4-channel thromboelastograph. Plasma fibrinogen was tested by the method of R. A. Rutberg; Willebrand's factor was tested by ristomycin aggregation of formalin-fixed thrombocytes using a microtitration technique; induced aggregation of thrombocytes was tested by the method of Born—O'Brien on a Sienco (USA) aggregometer. Freshly prepared ADP and epinephrine (A) solutions in an end concentration of 10^{-5} M were used as inducers. We assessed vascular hemostasis according to change in blood thromboxane and prostaglycine, and according to their

stable metabolites, thromboxane B_2 (TxB_2) and 6-ketoprostacycline $F_{1\alpha}$ (6-keto-PG $F_{1\alpha}$) by the method of radioimmunological analysis (using the commercial kits of Seragen, USA). Blood samples were drawn into silicon-coated test tubes with freshly prepared solutions of indomethacin and EDTA, and they were placed in an ice bath. The tubes were centrifuged at —4°C temperature. The samples were processed within the first 30 min after drawing blood. Extraction was performed with ethyl acetate.

Table 1. TEG parameters of subjects with neurocirculatory dystonia submitted to "dry" immersion

Parameters of plasma hemostasis	Before immersion (baseline)	Day of immersion 3	Day of immersion 7	Recovery period (5th day)
R, min	4.98±0,22	3,35±0,16*	2,53±0,13**	3,96±0,41
K, min	2,42±0,21	2.22±0,21	2,34±0,17	2,40±0,23
ma, mm	53,0±2,10	55,4±1,98	57,8±1,97*	54,4±2,09
E, %	130,01±8,05	141,0±7,81	154,2±7,12*	133,3±8,21
t, min	13,35±0,52	10,12±0,44	14,2±0,15*	14,0±0,14
S, min	16,73±1,04	12,1±0,93*	17,35±0,72	16,0±1,5
T, min	19,37±1,02	14,73±0,71*	24,13±0,95*	19,3±1,8
<α, degrees	13,2±0,92	15,17±0,98*	12,5±0,96	13,0±0,95
CR, %	5.4±0,20	4,26±0.28	4,38±0,33	4,3±0,4
FAB, %	20,01±0,40	14,67±0,17*	13.22±0,96**	15,7±1,0
F, mg%	295,9±10,51	336,18±11,04	390.0±15,42*	350,3±14,7
WF, %	121,6±11,4	168,2±12,34*	133,7±12,3	112,1±10,2

* $p<0,05$.
** $p<0,001$.

Results and Discussion

The parameters of plasma hemostasis before immersion were generally within the normal range (Table 1.). Three days after immersion in the tank there were distinct signs of activation of clotting activity and diminished activity of the blood anticolagulatory system. Thus, there was shortening of indexes R (reaction time) and T (total blood-clotting time), increase in E (elasticity of clot), decline of indexes CR (retraction of clot) and FAB (fibrinolytic activity of blood), some increase in concentration of fibrinogen (F) and elevation of Willebrand factor (WF) level. On the 7th day of immersion there was dissociation of coagulation parameters: ma (maximum amplitude of clot) remained increased, R diminished, F concentration continued to increase ($p<0.05$) and FAB diminished. Other parameters came close to the normal levels (indexes t, S and T grew longer) and WF began to decline ($p<0.05$).

Five days after termination of immersion, most parameters were still somewhat above normal. These changes in plasma hemostasis were indicative of diminished adaptive function in this group of individuals, since signs of hypercoagulation persisted in them even after stopping the immersion sessions.

The parameters of thrombocyte hemostasis changed concurrently with the tests for plasma hemostasis. Thus, before immersion all parameters of aggregatograms were virtually in the normal range, with the exception of ristomycin aggregation, where we observed increased functional activity of thrombocytes (Table 2).

There was a tendency toward increase in functional activity of thromocytes for both ADP and E aggregation after 3 days of immersion, there being absolutely no

Table 2. Parameters of platelet aggregation and vascular hemostasis in subjects with neurocirculatory dystonia submitted to "drt" immersion

Immers. / days	ADP, 10^{-5} M			Platelet aggregation inductors A, 10^{-5} M				Ristomycin		Vascular hemostasis	
	ia	ta	id	td	ia	ta	id td	ia	ta		TxB_2
Baseline	38,5± 4,44	4,18± 0,65	18,33± 0,94	2,96± 0,78	32,66± 3,68	4,88± 0,56	Irrevers aggregat.	82,4± 6,54	2,94± 0,64	277,16± 32,12	466,66± 33,71
3d	40,4± 3,0	2,74± 0,33	Irreversible aggregation		35,20± 3,4	4,46± 0,86	Same	50,6±* 5,38	2,06± 0,21	—	—
7th	41,4± 2,15	1,92±** 0,75	Same		39,6± 3,7	2,98±* 0,98	» »	81,75± 8,23	2,02± 0,16	110,0±* 15,22	339,0± 36,7
	52,4±* 3,24	2,03±* 0,55			25,60±* 2,64	6,14± 0,58	» »	56,6±* 5,4	2,38± 0,32	166,5± 15,6	660,16± 42,05

Key: -) no tests made *p<0.05, **p<0.01

disaggregation. On the 7th day of immersion, aggregation was still reliably high, particularly for induction of ristomycin. After immersion, functional activity of platelets dropped to the baseline for induction of E and ristomycin, and it remained high for ADP induction. This is also indicative of partial changes in parameters of platelet hemostasis.

We studied parameters of vascular hemostasis in 6 subjects, before immersion, on the 7th day of immersion and 5 days after it.

Before the start of immersion, 6-keto-$PGF_{1\alpha}$ and TxB_2 were in the normal range, constituting 222.16 ± 32.12 and 466.66 ± 53.71 ng/ml, respectively. On the 7th day of immersion there was dramatic decline of keto-$PGF_{1\alpha}$ to 110.0 ± 15.22 g/ml ($p<0.05$), and TxB_2 dropped to 339.0 ± 36.17 ng/ml ($p>0.05$). Five days after immersion 6-keto-$PGF_{1\alpha}$ level began to rise and reached 166.5 ± 15.6 ng/ml ($p>0.05$), while TxB_2 rose to 660.16 ± 42.05 ng/ml ($p<0.05$).

A very definite correlation is demonstrable between parameters of platelet aggregation and vascular hemostasis. Before immersion the parameters of cellular and vascular hemostasis were in the normal range. As intensity of thrombocyte aggregation increased by the 7th day of immersion, there was a decline to almost half the former level in 6-keto-$PGF_{1\alpha}$, TxB_2 level remaining virtually unchanged. After immersion, by the 5th day of recovery, intensity of thrombocyte aggregation decreased to the baseline for epinephrine induction, but remained high for ADP induction. Parameters of vascular hemostasis underwent the following changes: 6-keto-$PGF_{1\alpha}$ had a tendency toward increasing, coming close to the baseline, while TxB_2 also increased and even exceeded the baseline.

It is known that 6-keto-$PGF_{1\alpha}$ and TxB_2 reflect reflect the levels of their precursors— prostaglycine and thromboxane A_2, which are directly involved in vascular hemostasis. According to the literature, thromboxane stimulates platelet aggregation

[7, 8], elicits spasms of vessels, bronchi and trachea [8]. Prostacycline has the exact opposite action; it is an active disaggregant [6], spasmolytic [5] and, according to initial data, it is an antiatherogenic agent [8].

In our studies, the increase in platelet aggregation on the 7th day of immersion occurred in the presence of dramatic decline of 6-keto-$PGF_{1\alpha}$ and insignificant decline of TxB_2. Gradual restoration of baseline intensity of thrombocyte aggregation in the recovery period coincided with increase in 6-keto-$PGF_{1\alpha}$. Since 6-keto-$PGF_{1\alpha}$ and TxB_2 are conjugate systems, a decrease or increase in the former led to changes in the latter as well.

Thus, the parameters of vascular, cellular and plasma hemostasis are closely correlated: decline of 6-keto-$PGF_{1\alpha}$ and rise of TxB_2 lead to increase in functional activity of thrombocytes, which leads to increase in thrombus-forming potential of blood. A decrease in blood flow rate, impaired vascular permeability, discharge of coagulants and vasoactive substances from formed blood elements could serve as a triggering mechanism leading to increased coagulability of blood in simulated weightlessness.

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SIGNIFICANCE OF NUTRITION TO CHANGE IN HUMAN CARBOHYDRATE AND LIPID METABOLISM UNDER EMOTIONAL STRESS

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 22, No 1, Jan-Feb 88 (manuscript received 28 Feb 86) pp 13-17

[Article by V. P. Bychkov, L. I. Mosyakina and O. S. Khokhlova]

[English abstract from source] Two experiments were performed on 16 test subjects (13 men and 3 women) to study stress effects on the blood content of sugar and cholesterol. The test subjects were given a nutritionally balanced diet of canned foodstuffs. The caloric value of the diet was adequate to energy expenditures. In the first experiment, the test subjects were also given vitamin E, nicotinic acid and other vitamins constituting the polyvitamin complex Aerovit. In the second experiment, they were additionally given calcium and potassium salts, glucose and phosphatide concentrate. The stress-agent was a test in the rotating chair in the first experiment and a psychologic test (mental work within a limited period of time to reach success or failure) in the second experiment. The content of sugar and cholesterol before and after the stress-effects did not differ significantly. This can be attributed to the prophylactic effect of the nutritional factor on carbohydrate and lipid metabolism in an emotionally stressed man.

[Text] At the present time, the opinion has been formed in the scientific literature [2, 5, 7, 10, 11, 13] that emotional stress is associated with appreciable changes in carbohydrate and lipid metabolism (increase in blood sugar, cholesterol and free fatty acids). These changes are an expedient response when stress is associated with considerable expenditure of energy, which is covered by mobilization of glucose and fatty acids. When emotional stress is not associated with significant physical labor, the excessive sugar and free fatty acids circulating in blood could have an adverse effect. With regulation of metabolism in a healthy individual, the above changes are generally quite brief in duration [8, 13, 16]. Thus, a significant increase in blood epinephrine and fatty acids has been demonstrated in motor car racers prior to starting and 1 and 15 min after a race [6]. One hour after the competitions, these parameters returned to normal levels. Triglyceride content reached a maximum 1 h after a race, then dropped. Cholesterol concentration did not change.

Data on changes in a number of biochemical responses during exposure to various stress factors have been summarized in the monograph of F. Z. Meyerson [7]. One of

Table 1. Glycemia level (g/*l*) of subjects in first study

Subject	Day of Study									
	1		3				5			
	rotation									
	I		I		II		I		II	
	bef.	after	bef.	after	bef.	after	bef.	after	bef.	after
R-in	1,20	1,11.	1,37	1,50	1,50	1,35	2,10	1,50	1,45	1,30
M-ov	1,47	1,20	1,42	1,30	1,40	1,30	1,25	1,32	1,17	1,20
A-ov	1,45	1,40	1,50	1,80	2,10	1,50	1,45	1,77	1,35	1,30
T-ova	1,70	1,47	1,47	1,27	1,40	1,40	1,65	1,52	1,60	1,45
Kh-ova	1,35	1,60	1,40	1,32	1,65	1,60	1,27	1,15	1,40	1,27
S-ova	1,42	1,42	1,25	1,20	1,40	1,35	1,10	1,20	1,00	1,05
I-in	1,25	1,65	1,05	0,90	1,10	0,97	1,17	1,42	1,45	1,32
S-ov	1,15	1,27	1,20	1,00	—	—	1,00	1,22	1,00	1,05
K-in	1,57	1,75	1,67	1,60	1,65	1,77	1,45	1,35	1,52	1,05
B-ov	1,45	1,60	1,95	1,80	1,27	1,72	1,50	1,40	1,15	1,35
M	1,40	1,45	1,43	1,37	1,50	1,44	1,39	1,38	1,31	1,23
±*m*	0,05	0,07	0,08	0,10	0,09	0,08	0,10	0,06	0,07	0,04
p	>0,05		>0,05		>0,05		>0,05		>0,05	

Data on changes in a number of biochemical responses during exposure to various stress factors have been summarized in the monograph of F. Z. Meyerson [7]. One of the effective means of correcting metabolic changes that occur in stress situations is to have a properly balanced diet, with which there is sufficient intake of physiologically active substances (vitamins, lipotropic agents, antioxidants, etc.). They are naturally occuring components of regulatory systems that provide for reversibility of adverse changes that occur under stress.

Most studies dealing with the effects of stress agents on metabolism did not involve strictly controlled diet. However, there are data in the literature concerning the normalizing effect on metabolic processes under stress of such nutrients as vitamins [2, 6], antioxidants [7], potassium salts [12] and phosphatides [1].

Earlier investigations [3] established that exposure to various stressors was not associated with changes in parameters of lipid metabolism when subjects were on a balanced diet containing supplemental amounts of phosphatides, some vitamins and minerals. At the same time, the subjects presented changes in carbohydrate, protein and vitamin metabolism.

Hypokinesia and being in small closed chambers have an effect on carbohydrate and lipid metabolism. Particularly marked changes are observed under the combined effect of stressors and inadequate motor activity [4, 14]. For this reason, a study was undertaken of the combined effect of these factors on human carbohydrate and lipid metabolism with inclusion in the diet of prophylactic nutrients.

Methods

Two studies were pursued on 16 volunteer subjects. We used satisfactor diets that were well-balanced in nutrients. Their caloric value was consistent with the energy expended by the subjects. In the first study, the diet consisting of sterilized and

Table 2. Lipid and carbohydrate metabolism in 2d study (M±m)

Parameter	Norm	Baseline				HDT								Recovery period			
		day of study															
		6		9		6		10		16		21		29			
		before	after	bef.	after	bef.	after	bef.	after	bef.	after	bef.	after	bef.	after		
Serum cholesterol mmol/ℓ	3,96± 0,31	3,96± 0,28	4,14± 0,41	3,98± 0,23	4,06± 0,34	3,88± 0,34	4,01± 0,31	4,32± 0,18	4,47± 0,23	4,47± 0,34	4,50+ 0,34	4,45± 0,23	4,65± 0,39	4,11± 0,28	4,16± 0,26		
Blood sugar, g/ℓ	0,94± 0.05	0,86± 0,04	0,69± 0,02*	0,95± 0,02	0,92± 0,03	1,08± 0,08	0,90± 0,04	1,16± 0,04	0,87± 0,05**	1,06± 0,07	0,88± 0,04	0,94± 0,08	0,94± 0,05	0,86± 0,05	0,86± 0,06		

* $p<0,01$.
** $p<0,002$.

dehydrated foods contained 3121 kcal (13,058 kJ), with 113 g protein, 133 g fat and 393 g carbohydrates. In the second study, we used a diet of dehydrated, sterilized canned and deep-frozen products, with analogous levels chemical substances to those used in the first study. In the baseline and recovery periods, the subjects were on a diet containing 3127 kcal (13,075 kJ) with 135 g proteins, 127 g fat and 385 g carbohydrates. During the period of antiorthostatic (—8°) hypokinesia (HDT [head-down tilt]), the diet was reduced to 2625 kcal (10,983 kJ) with 115 g protein, 106 g fat and 323 g carbohydrates.

To enhancee adaptability, the subjects received a supplement of 40 mg vitamin E, 30 mg nicotinic acid and other vitamins, contained in the multivitamin product, Aerovit, in the first investigation. In the second one, they used the same vitamins, but ascorbic acid was increased to 300 mg. In addition, the subjects took 1 g potassium, 270 mg calcium, 60 g glucose (30 g during HDT) and 4 g phosphatide concentrate (daily).

Seven healthy male subjects 26 to 31 years of age and 3 women (T-ova, Kh-ova, and S-ova) 28 to 47 years old participated in the first study. The subjects were exposed to a stressor, in the capacity of which rocking in a revolving chair was used, on the 1st, 3d and 5th days in the sealed chamber. Blood sugar concentration was measured before and after rocking.

Six men 26 to 33 years of age participated in the 2d study. They spent 58 days in the hospital, including 28 days (baseline) without restriction of movement (baseline), 14 days (experimental period) of HDT (—8°) and 26 days (recovery period) during which they adhered to a graded exercise program, received hydrokinesitherapy under water and general fortifying agents.

The subjects were exposed to a stress agent, which consisted of a psychological test (time-limited intensive mental activity using "success" and "failure" situations), on

the 6th and 9th days of the baseline period, 6th, 10th and 15th days of HDT and 21st and 29th days of the recovery period.

Carbohydrate and lipid metabolism was examined using blood drawn from the finger and vein before use of the stressor and immediately after it. Blood sugar was measured by the anthrone method [9] and serum cholesterol by the method of Huang et al. [15].

Results and Discussion

Table 1 lists data on dynamics of blood sugar in the first study. There were considerable individual fluctuations in the subjects' responses to stress. We failed to observe changes in parameters in the same direction under the influence of stress. Mean data for 10 subjects following stress did not differ reliable from values obtained before exposure to stress.

Table 2 lists data on carbohydrate and lipid metabolism obtained in the second study.

Values for these parameters obtained on fasting subjects whose motor activity was unrestricted (baseline period) without stress situations are listed in the "Norm" column.

With use of stress in the baseline period (6th and 9th days), no statistically reliable elevation of serum cholesterol or blood sugar was demonstrable.

There was a tendency toward increase in serum total lipids and cholesterol, decrease in α-cholesterol and α-lipoproteins, extension of hyperglycemia time after sugar load and elevation of fasting blood sugar ($p>0.05$ for all parameters). However, there was no reliable elevation of cholesterol or blood sugar in any case of stress during HDT (6th, 10th and 15th days). In the recovery period, blood cholesterol and sugar levels did not change under the effect of stress factors (see Table 2).

Throughout the period of the investigation, the parameters of lipid and carbohydrate metabolism obtained following stress were in the physiological range, and they did not exceed mean values obtained on fasting subjects in the baseline period, when serum cholesterol was 3.96 ± 0.31 mmol/ℓ; after exposure to stress (mean data for 6 subjects in 7 stress situations), the figures were $4.29 - 0.13$ mmol/ℓ ($p>0.05$). Blood sugar values were 0.94 ± 0.05 and 0.88 ± 0.02 g/ℓ ($p>0.05$).

During our studies, none of the stress situations caused noticeable elevation of blood sugar and cholesterol, although other researchers [2, 5, 7, 8, 10, 11, 13] have observed changes in these parameters under emotional stress when the diet was not controlled.

Thus, when using diets that are balanced in essential nutrients with vitamin (including antioxidant) supplements and other substances that enhance adaptation to stress, we did not observe noticable elevation of blood cholesterol and sugar in stress situations with ordinary motor activity or under hypokinetic conditions.

All this is indicative of the fact that the nutritional factor is of some relevance to regulation of carbohydrate and lipid metabolism under emotional stress.

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ANALYSIS OF CLINICAL SYMPTOMS OF HUMAN DECOMPRESSION SICKNESS DURING ALTITUDE CHAMBER STUDIES

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 22, No 1, Jan–Feb 88 (manuscript received 16 Sep 86) pp 17–21

[Article by L. R. Iseyev, A. S. Tsivilashvili and V. I. Chadov]

[English abstract from source] Over 2400 altitude chamber ascents in which 130 volunteers participated were performed using different decompression tables. The cases of decompression disease were classified in terms of its types and severity. It is stressed that the experimenters involved in decompression studies have to be extremely careful because the disease may have various and sudden manifestations.

[Text] The diversity of forms of manifestation if altitude decompression sickness (ADS) has made it necessary to classify them. We feel that the most acceptable classification of these disorders is according to localization and severity. Thus, the following basic forms have been determined according to localization of symptoms [1—4, 6]: musculo-articular (50—90% of the cases), cutaneous (10-24%), pulmonary (2—3%) and neurocirculatory (1—2%).

A distinction can be made of four degrees of severity of decompression sickness which consider the dynamics of development of ADS [4]: 1) mild, transient, unpleasant sensations of diverse localization, including the cutaneous form, hyperesthesia and paresthesia (if they are the only symptoms); 2) moderate pain in the region of muscles, joints and bones which do not prevent performance of physical work; 3) intense, rapidly progressing pain of any localization which prevents performance of physcial work; 4) severe local or generalized disorders that require medical care (collapse, asphyxia, sluggish and spastic paralysis, distrubances referable to the CNS [central nervous system], acute autonomic system functional disturbances). Our objective here was to submit a summary description of ADS cases observed in the course of many years of investigations, in accordance with the existing classification of disorders as to localization and severity, and to illustrate from this sample the most typical cases of ADS.

Methods

A total of 2444 "altitude" studies under different conditions of decompression were conducted between January 1975 and August 1986 on 130 volunteer subjects. Since

the main objective of these studies was to explore the probability of onset of ADS, its incidence, localization and severity, as well as to validate safe transitions to low pressure with respect to ADS, the "ascents" were made with or without prior desaturation at differeent "altitudes," but with mandatory breathing of oxygen or an oxygen-nitrogen mixture with no more than 12% nitrogen.

Results and Discussion

In these studies, we found 250 cases of ADS (10.2%), in 5 of which (2.0%) it was the pulmonary form, 4 (1.6%) were the neurocirculatory form, 22 were the cutaneous form (in 3 cases combined with the musculo-articular form, 8.8%) and 219 (87.6%) were the musculo-articular form. Thus, the conventional proportions were approximately present. We should like to call attention to the course of some special cases of ADS.

In the series of studies where ascents to 7000 m were made daily for 6 days while breathing oxygen with 10-12% nitrogen, subject Ch. complained of vertigo on the 4th day while performing work (in 5th min of climbing up a step). He was asked to stop working. The vertigo disappeared rapidly, and he asked permission to continue with the study. Heart rate (HR) and respiration rate (RR) constituted 63 and 18/min, respectively, prior to his complaint. In the 11th min (according to the subject) after stopping work he began to heave. After a rapid "descent" to 5000 m the subject began to vomit. The descent was continued. Vomiting recurred on the "ground" 3-5 min after exiting from the altitude chamber. Examination revealed complaints of general fatigue, vertigo (objects floated to the left), which was more severe upon movement, and mild nausea. Objectively, the following was found: no marked disturbances of some cortical functions; pupils D=S; active response to light; distinct nystagmus with slight rotational acomponent when looking to the left; transient diplopia when looking to the left; slight flattening of right nasolabial fold; slight deviation of the tongue to the left; full range of limb motion; tendon and periosteal reflexes active for upper and lower extremities D=S; no pathological sings; sensibility intact. The focal neurological symptoms with "acute" onset were evaluated as a dynamic cerebrocirculatory disturbance in the vertebrobasilar region due to decompression disorder.

Further in-hospital observation revealed regression of focal symptoms, which disappered entirely within the next 3—5 h. Since the subject did not present with such symptoms as pain, itching, cough, asphyxia prior to worsening of his condition, it can be assumed that, in this case, we were dealing with the neurocirculatory form of ADS. The subject was discharged from the clinic in good condition after 3 days, and he subsequently participated many times in various investigations. It must be stressed that this was the most serious case of cerebral ADS in our practice.

There was another, less graphic and typical, "discrete" case of neurocirculatory ADS, which occurred in a frequent participant of altitude tests, N-v; in the 20th min at "high altitude," during work, the physician noticed progressive bradycardia in this subject (HR dropped from 80 to 40/min). When asked how he felt, the subject reported sudden onset of weakness, sensation of faintness and heat over the entire body, after which he began to perspire profusely. In view of his precollaptoid state, he was asked to stop working and remain seated. As he rested, the subject's condition improved rapidly and all of the above signs disappeared in 15 min. At his request, work was continued and he completed the entire program. He presented no more complaints to the end of the study. After the test, the subject merely reported some fatigue. Examination at the hospital by a neurologist failed to demonstrate any deviations.

M-n was particularly sensitive and susceptible to ADS; during exposure to relatively mild decompression he repeatedly demonstrated primarily the cutaneous form of ADS of diverse localization, characterized by marked pruritus, which disappeared during "descents" and vivid "blooming" erythema that persisted on the "ground" as well for several hours. In the 120th min at an "altitude" of 9300 m, while working on special manual exercise equipment, this subject complained of a scratchy throat and infrequent cough. These signs disappeared after 20-25 min, and the subject continued to perform the test program. In the 242d min at "high altitude," he complained of severe pain in the right knee. "Decompression" was performed after 4 min and he "descended" to the "ground." During pressure elevation, the pain disappeared at 4000 m. After the descent, the subject reported that insignificant pain appeared in both knees in the 3d h at "high altitude,." after which, by the end of the 3d h, he developed pinpoint pain in the right jaw. The painful point shifted up toward the temporal region within 2-3 min. He experienced a brief black-out, after which all of the unpleasant sensations in the head region disappeared. While at "high altitude" he did not report any of this to the physician.

On the "ground," after removal of electrodes and changing his clothes (about 10 min after the "descent), the subject experienced severe vertigo and weakness. On his way to the physician he swerved to the right and almost fell. After resting for 40 min (during which time he breathed pure oxygen), all of the above signs disappeared. External examination revealed hyperemia in the middle third of the right arm, on the lateral aspect, 5×10 cm in size, and isolated vesicular eruptions. M-n, escorted by a medical worker, was referred to the clinic for in-hospital examination and observation. There, examination revealed moderate blemishes, some increase in pulse and respiration rates; arterial pressure (BP) was 105/80 mm Hg; his consciousness was clear and there were no cortical functional disturbances; pupils D=S, active reaction to light, mild nystagmus with a rotatory component when looking to the right; no paresis; tendon and periosteal reflexes D=S, active; no pathological pyramidal signs; satisfactory performance of coordination tests. The demonstrated changes were indicative of cerebrovascular spasm with the syndrome of vertebral circulatory insufficiency. After 14 h all these signs disappeared, and the subject was discharged in good condition.

In 3 studies pursued to define the thresholds of decompression gas production by means of ultrasonic doppler equipment, unpleasant sensations appeared in the throat, tickling feeling, occasional cough and some retrosternal discomfort, i.e., symptoms inherent in the pulmonary form of ADS, were observed in 1 subject at 8800 and 9100 and in another at 8200 m, 2-4 h after resting in seated position. When pressure was raised, the symptoms of the first subject disappeared at residual pressure levels of 504.0 and 433.3 gPa, respectively, and in the other—on the "ground," which is also typical of ADS in general (with the exception of the cutaneous form). Control gas analysis of the contents of the oxygen tube on a mass spectrometer revealed that the mixture contained over 99% oxygen and about 1% inert gases. Another case of the pulmonary form of ADS was described above (subject M-n).

The following case is a vivid example of the cutaneous form of ADS. The subject, who was a physician, had participated in diverse types of altitude tests for 10 years and never before had any manifestations of ADS. He also participated in a group study, the purpose of which was to determine the efficacy of 2-h decompression at residual pressure of 293.3 gPa. The climb to 9200 m began with concurreent breathing

of pure oxygen, decompression at "high altitude" was performed for 2 h at rest, after which the subjects were to perform step, bar and cycle ergometer exercises for 4 h.

By the end of the 1st h at an "altitude" of 9200 m, the above-mentioned subject reported pruritus of the right gluteus. Later on he developed numbing and cutaneous paresthesia in the same region. These signs were evaluated as being the result of sitting for a long time. In the 130th min at "high altitude" he developed puritus on the abdomen in areas where the electrode tape adhered firmly to the skin. Examination revealed, in addition to "goose flesh," diffuse hyperemia of the abdominal skin (overall involvement of the umbilical region, with some irregularly shaped areas on the periphery). Hyperemia involved the region from the costal arch to the anterior iliac bones. The skin was also pasty. In the 147th min at "high altitude," while performing step exercises, there was intensification of abdominal pruritus, but no changes in the gluteal region.

Blood was drawn from this subject, and his "descent" was begun in the 165th min. Pruritus disappeared at 677.3 gPa. One hour after "descent" to the "ground," the marbly pattern was still present on the abdomen and right gluteal region (pinkish-cyanotic). In addition, we found diffuse eruption (erythema) over the entire left axillary region. After 3 h, we observed edema of abdominal skin (significant) and buttock (to a lesser extent), the color of the marbled pattern became generalized, the cyanotic regions being slightly indented. There was no pain. One day after the "climb," edema of the abdominal skin increased, while the right buttock showed a 1.5-fold increase in size, as compared to the left. Palpation revealed tenderness, not only of the integument of the anterior abdominal wall, but in deeper lying tissues, as well as in the region of attachment of thoracic and pelvic muscles. The marbly skin pattern began to disappear. Two days after the "climb," all of the above signs disappeared. Thus, as a result of decompression there was massive involvement of subcutaneous tissue of the abdomen, left axillary region and right buttock, with development of erythematous eruption and edema.

As can be seen from this example, the cutaneous form of ADS, which is generally considered a mild manifestation of decompression sickness, was more complicated in this case.

In all of the other (221) cases of musculo-articular form of ADS of any localization (including polytopic) and severity occurred in accordance with the standard classical model with appearance of the local pain syndrome of varying severity and disappearance of pain as pressure was raised to that of the ground. In these subjects, no other clinical manifestations indicative of local ADS were demonstrable after their "descent" or on the days that followed. There were merely some signs of expected fatigue from the study with reference to physiological functions.

In this time, 2 subjects (U-v, S-y) presented with barotitis in the form of heavy-headed feeling, congested ears and transient earache (sensation of compression). During the "descent," this pain at first increased, but disappeared at about 5000 m. Examination revealed that the tympanum of the involved ear (on the left in both cases) was retracted, vessels were injected, and subject U-v showed minimal petechia on the tympanic membrane. All these symptoms disappeared in 18-25 h (after inflation of the ears, massage of the tympanic membrane and inhalation).

Analysis of composition of peripheral blood of some subjects before and after ADS revealed that there were no appreciable changes beyond the physiological range; however, we were impressed by the tendency toward decrease in hemoglobin, red blood cells and thromocytes, increase in reticulocytes. In the white blood cell formula, an increase in eosinophils was noted in 30% of the cases and in basophils in 21.6%. It should be noted that eosinophilia was observed more often with the mixed form (cutaneous and musculo-articular), and basophilia—with the musculo-articular form. In addition, plasma cells and myelocytes were demonstrable (up to 0.5—1%) in some subjects.

However, these changes in hemocytoblast system cells (decrease in hemoglobin and red cells with increase in reticulocytes) should alert us, since they could be indicative of hemolysis during "ascents" in an altitude chamber; this phenomenon must be thoroughly investigated by means of special tests for osmotic resistance of erythrocytes and pigment metabolism (bilirubin and urobilin). As for the increase in eosinophils in the leukocyte formula, this change can be explained as follows. It is known that in the presence of pain in general, probably including altitude-decompression pain (as well as in cutaneous manifestations of ADS associated with pruritus, edema, etc.), there is accumulation of nociceptive "algogenic" substances [2], histamine and histmainoid compounds combined under the general name of kinins, at the sites of involvement. These kinins have an allergy-producing action, and for this reason the concomitant eosinophilia could be an objective, though indirect, indication of ADS.

These were the most exquisite cases of ADS in the entire sert of our studies. They are indicative of the virtually unlimited diversity of forms of these disorders, which can sometimes be quite serious and hazardous to human health. This diversity of forms and suddenness of manifestation of disorders compel even highly qualified personnel involved in such altitude investigations to be extremely careful and alert.

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ELECTROENCEPHALOGRAPHIC CHANGES DURING EQUILIBRIUM TEST IN THE PRESENCE OF RHYTHMIC PHOTIC INTERFERENCE

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 22, No 1, Jan–Feb 88 (manuscript received 10 Mar 87) pp 21–25

[Article by Ye. T. Petrenko]

[English abstract from source] Reliable diagnosis of CNS noise resistance is very important in the selection of operators, pilots and cosmonauts. This dictated a study the purpose of which was to investigate neocortex biopotentials of 74 test subjects during equilibrium tests in the presence or absence of 123 Hz light flashes. Electrocardiographic and stabilographic recordings were taken from 6 sites of the left neocortex during equilibrium tests (standing on toes) and during light stimulation. EEG's were processed through correlation-spectral analysis by means of Electronika-60 and EC-1035 computers. During light stimulation 35 "nonsusceptible" subjects maintained equilibrium for as long as 80–100% of the normal time, while 39 "susceptible" subjects maintained it for only 10-30% of the normal time. In response to light stimulation susceptible subjects showed distinct rearrangement of the autospectral and coherence functions. The spectral density increased by 115 –170% and the coherence of biopotentials that corresponded to the light stimulation frequency grew by 42–70% ($p<0.01$). Peaks of the function maxima occurred at 12 Hz. At the same time the density and coherence of biopotentials in the frequency range 6–8 Hz decreased by 50-58% and 15–28%. respectively ($p<0.01$). The above changes were more pronounced in the neocortex areas related to movement organization, viz. premotor, motor and sensorimotor areas. In the nonsusceptible subjects light stimulation induced no changes in EEG. It is concluded that noise resistance of the motor control system depends on the CNS capacity to prevent the rhythm of light stimulation to occur in EEG's of motor areas.

[Text] Evaluation of CNS resistance plays a large part in professional screening of individuals whose work will be performed under extreme conditions (pilots, operators, cosmonauts).

We previously [5–7] reported the results of our studies of the effect of rhythmic photic interference [light flashes] on time and space organization of bioelectric

potentials of the human neocortex and biomechanical movements. It was established that performance of movements in the presence of flashes at a frequency of 12 Hz is associated with appearance of motor regions in the neocortex with "slave" activity on the working electroencephalogram (EEG) in the rhythm of frequency of photic stimulation and decline of biomechanical efficiency of movements.

At the same time, the degree of such changes is apparently determined by individual distinctions of the movement control system. Our objective here was to determine the distinctions of time and space organization of neocortical bioelectric potentials in individuals differing in extent of decline of biomechanical effect of movements in the presence of rhythmic photic stimulation at a frequency of 12 Hz.

Methods

A total of 372 subjects 19–23 years of age participated in this study. Balancing on one toe—a biomechanically difficult exercise that is not associated with muscular artefacts (EEG)—was used as the motor model. Of this number of subjects, 74 were selected as a result of prior testing, and they made up 2 groups. The 1st group consist of "stable" subjects, in whom the flashes elicited minimal impairment of equilibrium and decrease in balance-holding time (by only 20%, as compared to ordinary conditions). The 2d group consisted of "unstable" subjects, in whom equilibrium lasted for 70=-90% less time.

All of the subjects performed the equilibrium test under ordinary conditions and in the presence of flashes generated by photostimulators at a frequency of 12 Hz and with brightness of 30,000 lux. During the exercise we recorded the EEG from 6 parts of the neocortex and stabilograms. Electrical activity was derived monopolarly from the projection on the head of the frontal, premotor, sensorimotor, inferoparietal and visual regions of the left hemisphere, as well as motor representation of muscles of the supporting (right) leg.

Cup-shaped niobium electrodes were attached to the head with collodion [7, 9]. Segments of EEG and stabilograms lasting 2-6 s, at a 17-ms sampling rate, were converted into variable-sign series and inputted in an ES-1035 computer. An Elektronika-60 microcomputer was used for quantization and primary conversion of recorded processes into digital form. Fourier transform of correlations between each pair of recorded processes was used to calculate the coefficients of paired correlation, autospectral and cross-spectral functions, as well as coherence. In all, we analyzed about 8000 such functions.

Results and Discussion

In subjects referable to the 2d group, performance of the complicated equilibrium test in the presencee of photic stimulation was characterized by significant and reliable changes in bioelectric potentials in all EEG leads. There were subtantial changes in autospectral functions. There was a dramatic increase in share of waves with frequency of 12 Hz ($p<0.01$). The density of bioelectric potentials corresponding to the frequency of flashes was 215–270% of the value under ordinary conditions. Peaks of maximal frequency spectra in the presence of flashes were at the indicated frequency, whereas under ordinary conditions they are in the range of 6–10 Hz. Maximum increase in density of bioelectric potentials with 12-Hz frequency was observed on the EEG of the left premotor region (270%). At the same time, in the presence

of photic stimulation there was dramatic, 50–58% decline of spectral density of EEG frequencies in the range of 6–8 Hz (Figure 1).

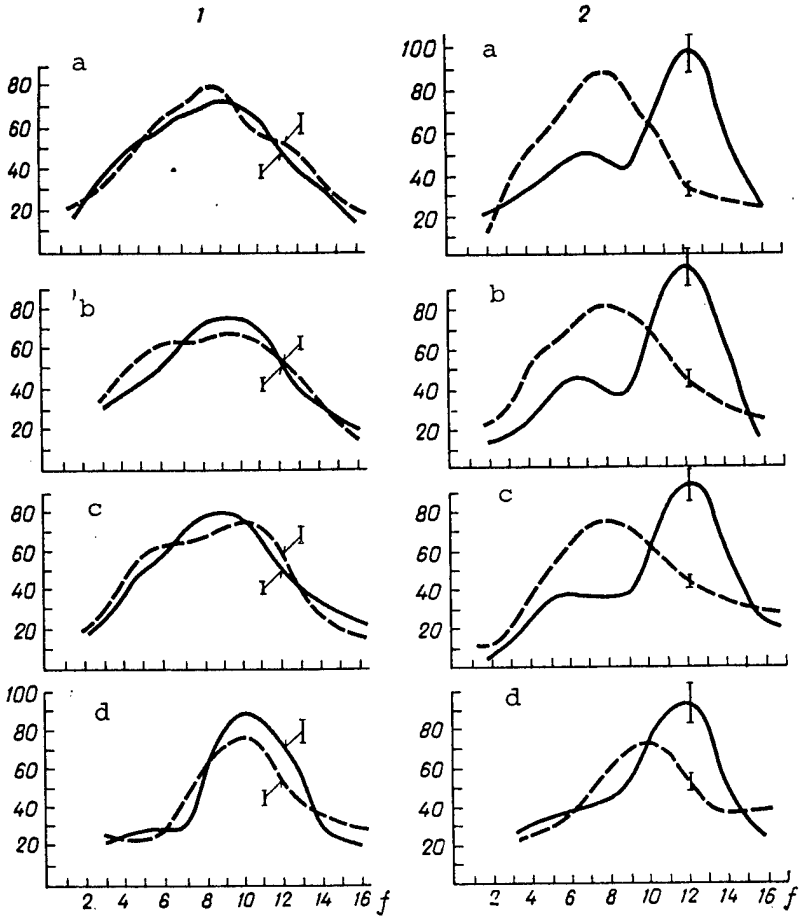

Figure 1. Autospectral functions of EEG of left premotor region (a), motor representation of muscles of the right leg (b), left sensorimotor region (c), and visual region (d) during equilibrium test under ordinary conditions (dash line) and in presence of photic stimulation (boldface line)
X-axis, frequency (Hz); y-axis, spectral density (relative units)
1 and 2) 1st and 2d group of subjects, respectively

We observed marked changes in coherence function. In particular, there was significant increase in coherence of EEG waves at 12-Hz frequency ($p<0.05$). Peaks of maximum coherence shifted to this frequency in all tested EEG pairs. On the average, coherence of bioelectric potentials corresponding to the frequency of the flashes increased by 42%, and its values were in the range of 0.40–0.60 (averages for 15 pairs of EEG's in the group of 39 subjects). Maximum increase (by 70%) was observed on the EEG of the frontal region and motor representation of muscles of the supporting leg (Figure 2). On the whole, in the subjects of this group autospectral functions and coherence in the presence of photostimulation were characterized by prevalence and coincidence of maximums at the frequency of photic stimulation.

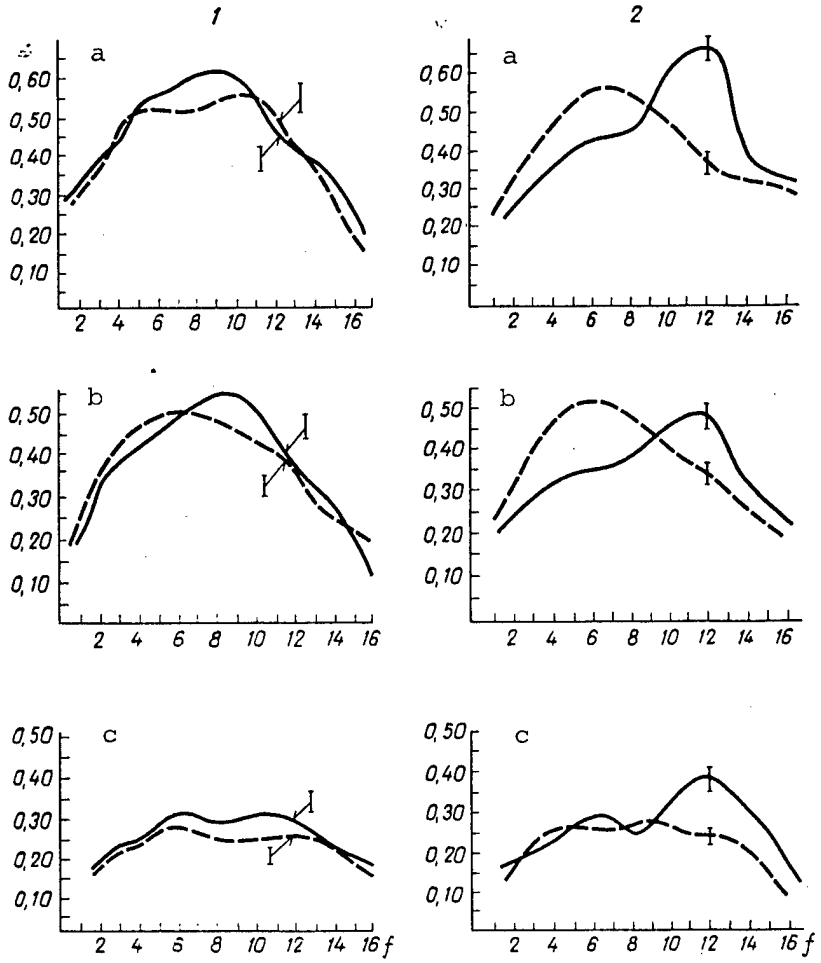

Figure 2. Coherence functions of EEG's of the left premotor region—motor representation of the right leg (a), left sensorimotor region—motor representation of muscles of the right leg (b), frontal and visual regions (c) during equilibrium test under ordinary conditions (dash line) and in the presence of photic stimulation (boldface line)

X-axis, coherence; y-axis, frequency (f) in Hz

At the same time, photic stimulation did not lead to reliable EEG changes in the 1st group of subjects, as compared to the EEG recorded during the equilibrium test under ordinary conditions. Thus, growth in density of bioelectric potentials corresponding to the frequency of flashes reached only 9–31%. There was 31% increase in share of these frequencies only on the EEG of the visual region. It was not observed in the motor and sensorimotor regions, whreas it constituted only 12% in the premotor region and was unreliable ($p>0.05$). The peaks of maximum frequency spectra during photostimulation were in the range of 6–10.5 Hz in this group of subjects, and the flashes had no reliable effect on density of 6–10–Hz frequencies ($p>0.05$). Coherence of the studied EEG pairs in the presence of photic stimulation did not differ from that recorded under ordinary conditions in the 1st group of subjects. Peaks of maximum coherence did not correspond to frequencies of photic stimulation, and they were in the range of 6–10 Hz.

Thus, rhythmic photostimulation at a frequency of 12 Hz had a dissimilar effect on the EEG of subjects in different groups. In subjects with significant decline of biomechanical effectiveness of equilibrium, flashes elicited appearance on the working

EEG of a significant number of "slave" waves corresponding to the frequency of photostimulation, which prevailed in all leads. Significant restructuring was noted in the EEG derived from the neocortical regions functionally responsible for performance of movements (premotor, motor and sensorimotor regions of the left hemisphere).

It was previously [7] reported that flashes of light at a frequency of 12 Hz had an analogous effect on the working EEG and diminished biomechanical effectiveness of balancing on a toe among most people who were not differentiated according to resistance to photic interference. It was stressed that, as a result of imposing the rhythm of photostimulation on the EEG there was impairment of optimum time and space relations between bioelectric potentials of premotor, motor and sensorimotor regions, which led to disorders in processes of controlling stability of the body on a small support.

It is known that the motor and sensorimotor regions of the neocortex implement the dynamic structure of motion, and premotor regions are involved in its rhythmic organization [1, 4].

As shown by analysis of our findings, flashes elicited more profound changes in time and space organization of bioelectric potentials of nerve centers functionally responsible for implementation of movements in the 2d group. In particular, they led to dramatic decline of density and coherence of bioelectric potentials in the 6–8 Hz range derived from premotor, motor and sensorimotor regions. On the basis of our data and prior reports [6, 7] it can be assumed that frequency–phase interactions between bioelectric potentials of regions whose activity is manifested at 6–8 Hz apparently play a significant role in intercentral integration which controls stability on a small support. Thus, as a result of total imposition by photic stimulation of "interferential" waves there is a decrease in share of "useful" functionally significant frequencies, which is what makes processes of intercentral interaction difficult.

At the same time, in the 1st group of subjects, in whom photic stimulation at a frequency of 12 Hz virtually failed to diminish biomechanical effectiveness of equilibrium, there were few "slave" waves on the EEG. Typically, they were virtually absent from the EEG of the motor, premotor and sensorimotor regions. In the presence of photic stimulation we did not observe reliable decrease in density of 6–8 Hz frequencies on the EEG of these neocortical zones.

On the whole, the relative stability of frequency and phase relations between bioelectric potentials with rhythmic photostimulation at 12 Hz is associated with optimum time and space interactions [2, 3, 8]. between nerve centers functionally responsible for performance of movement and intercentral integration processes that efficiently implement biomechanical programs.

The findings warrant the belief that resistance of the system that controls human movements to the disrupting effect of rhythmic photostimulation is determined by the capacity of the CNS to counteract appearance of "imposed" rhythm of flashes on the EEG by means of blocking it in the neocortical regions responsible for organization of movement. Such stability can characterize, to some extent, the noise resistance of the CNS.

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GROWTH AND DIFFERENTIATION OF CELLS IN ORGANOTYPICAL RAT EMBRYO CEREBELLAR CULTURE DEVELOPING IN WEIGHTLESSNESS

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 22, No 1, Jan-Feb 88 (manuscript received 5 Aug 86) pp 25–29

[Article by I. V. Viktorov, N. A. Shashkova, A. Privat and M.-J. Drian (USSR, France)]

[English abstract from source] Cerebellar cells of 18-day rat fetuses that developed for 5 days on Cosmos-1514 and those of synchronous and vivarium controls were cultivated for 21 days in Maximov chambers. Light microscopic examinations of live explants and semithin sections revealed no disorders in histotypical structures of explants or cytopathological changes in Purkinje cells and granule cells. It is concluded that space flight effects on the cerebellar morphogenesis of rat fetuses exposed to microgravity during days 13 to 18 of their prenatal development did not lead to such changes in the differentiation of nerve and glia cells which would cause morphogenetic disorders during postflight organotypical cultivation.

[Text] The purpose of the Soviet–French Purkinje–2 experiment, which was performed within the program of the embryological experiment aboard the Soviet biosatellite, Cosmos-1514 [2], was to investigate the effects of spaceflight factors on potential of Purkinje cells, granule and glial cells for growth and differentiation in postflight organotypical cultures of cerebellar explants. Such formulation of the purpose of this investigation was motivated by the results of studies of the cerebellum with genetic or experimental developmental disorders of its cellular structures at the early stages of embryogenesis, which revealed that such disturbances are manifested by distortion of processes of neuronal and glial differentiation and cultivation conditions [5, 8, 9]. The protocol of the Purkinje-2 experiment was also based on the assumption that further development, following a spaceflight, of the rat embryo cerebellum in an organotypical culture would preclude the postflight corrective influence of regulatory systems of the organism and would permit demonstration of morphological changes arising under the influence of weightlessness. The definite advantage of using tissue cultivation methods in such studies is that it is possible to make an *in vivo* study of development of cerebellar cell structures followed by morphological analysis of cultivated nerve tissue [6, 7].

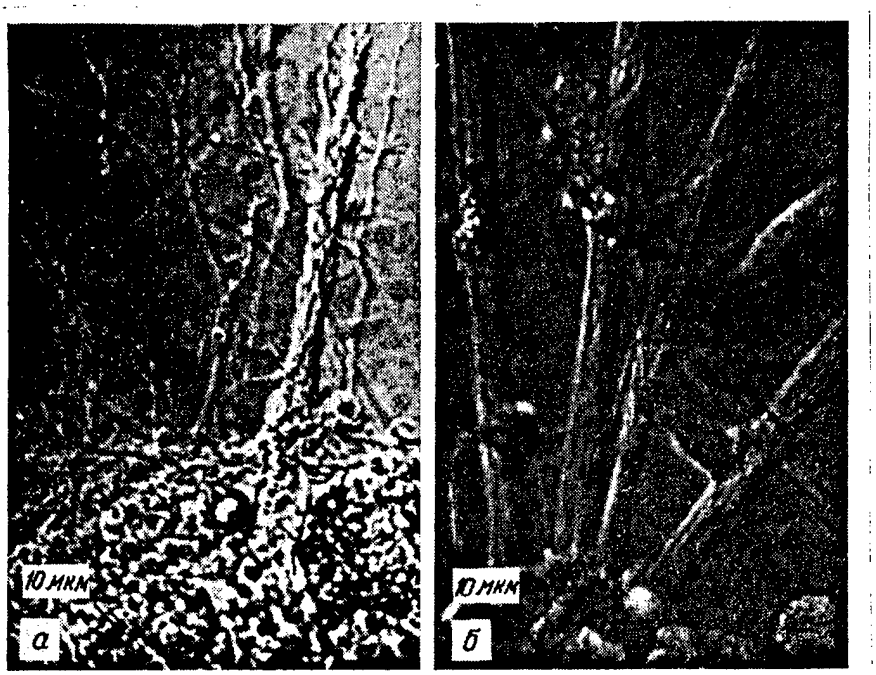

Figure 1. Initial fiber growth from marginal zone of explants of FG group of rat embryo cerebellum

In vivo microphotograph; scale 10 μ

а) isolated axon growth, 4 days of cultivation

б) glia-axon bundles, 7 days of cultivation

мкм) microns

Methods

The cerebellum from each 5th pair of 18-day embryos of 5 Wistar rats flown in space for 5 days, from the 13th to 18th days of the gestation period, served as the material for our study. Experimental material was collected at the landing site. The rats were decapitated without anesthesia 4–8 h after touchdown. After dissecting the abdominal cavity, the cornua of the uterus were extracted and placed in sterile Petri dishes. Embryos were extracted from the uterus, decapitated, and their heads placed for 1 min in 70% alcohol, then washed twice in Simms' salt solution. The skull of the embryos was opened and cerebellum isolated under sterile conditions, which were provided by a portable unit with laminar flow of sterile air. To prepare explants, the cerebellum was cut into 7–8 parasagittal sections 0.3–0.5 mm thick, which were placed, 2 at a time, on a cover glass 22 mm in diameter, which was covered with collagen. The slides with explants were transferred into the wells of plastic containers, to which we added 0.3 m*l* nutrient medium containing 40% Eagle's minimum medium, 12.5% human placental serum, 12.5% calf embryo serum, 25% Simm's saline, 800 mg% glucose, 2 mM glutamine, 0.2 U/m*l* insulin and 0.5 m*l* solution of essential amino acids ($\times$100) per 100 m*l* medium. The containers were sealed and placed in a self-contained Cytos portable incubator which maintained a temperature of 37.0$\pm$0.5° temperature for transportation to Moscow. The cultures were removed from the wells of the plastic containers 36 h after isolation of cerebellar explants, and they were transferred in a Maximov chamber in a fresh batch of culture medium. During further cultivation, the nutrient medium was changed regularly every 3d day. Daily intravital observation of culture development and microphotography were performed using an inverted temperature-controlled MBI-13

microscope. We made an *in vivo* analysis of the following parameters of development of cerebellar explants: time of their adaptation to cultivation conditions, spreading of tissue and formation of growth and migration zones, sequence and rate of migration of glial and granule cells and growth of fibers beyond the explant, distinctions and rate of differentiation of Purkinje cells and granule cells, their localization in the cultivated cerebellum sections, onset of neuroglial interactions and formation of axonal myelin sheaths.

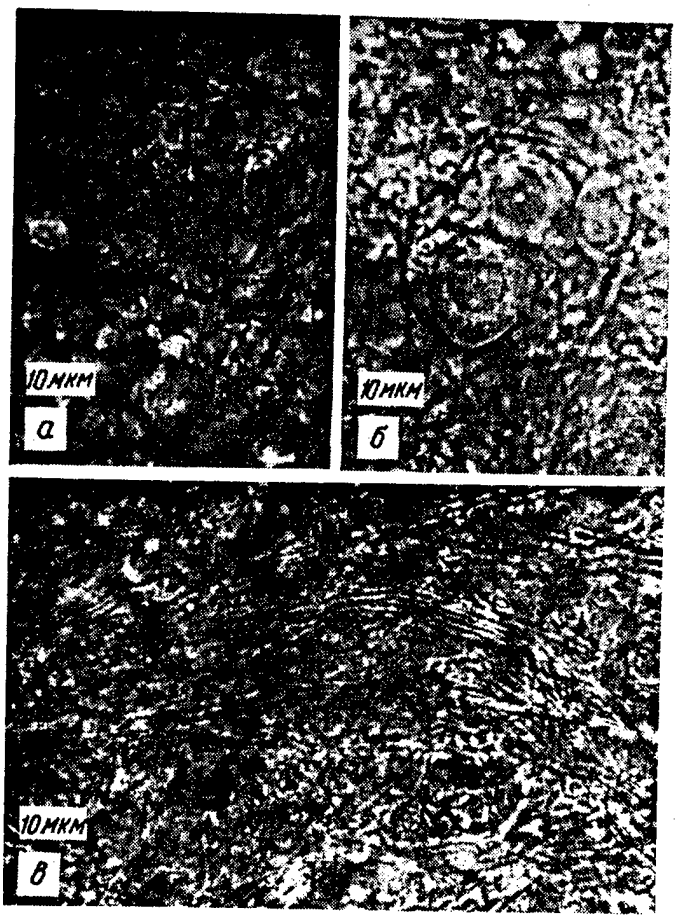

Figure 2. Cellular elements of explant of FG rat embryo cerebellum
In vivo microphotographs; scale 10 μ
а) granule cells of external granular layer, 12th day of cultivation
б) Purkinje cells in central zone of explant, 18th day of cultivation
в) myelinated axons in explant, 21st day of cultivation
мкм) microns

On the 21st day, the cultures on slides were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2), dehydrated in ascending concentrations of alcohol and imbedded in araldite. Semi-thin (1 μ) sections were stained with 1% toluidine blue prepared with 1% sodium tetraborate.

The cerebellums of rat embryos used in a synchronous ground-based experiment (SGE) and vivarium control (VC) were treated similarly for histological examination.

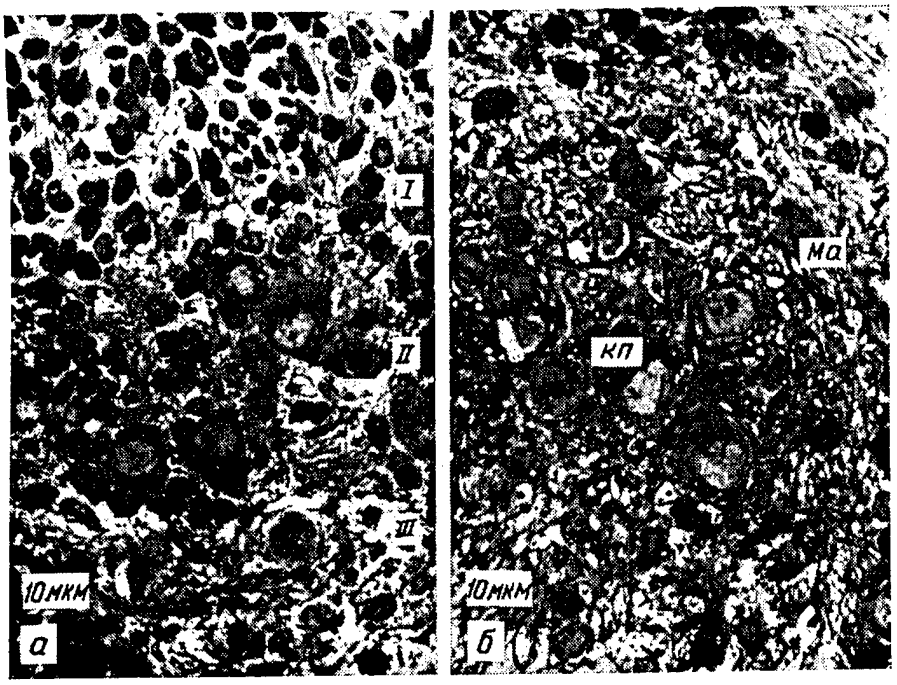

Figure 3. Cellular structure of explants of FG rat embryo cerebellum
Semi-thin (1 μ) sections, toluidine blue stain; scale 10 μ

а) cellular architechtonics of explant:
I) external granular layer
II) layer of Purkinje cells
III) neurons of cerebellar subcortical nuclei
б) Purkinje cells (кп) and myelinated axons (Ma) in central zone of explant

We examined a total of 53 explants of rat embryo cerebellum referable to the flight group (FG), 46 explants referable to SGE and 57 explants referable to the VC group.

Results and Discussion

In vivo observation of explant developed was started on the 3d day of cultivation. At this time we observed the start of growth of isolated zones from the marginal zone of 71% of the FG explants, 86.6% of VC explants and 81.6% of SGE explants (Figure 1a). Concurrently with fiber growth there was migration of individual glial elements and granule cells; 90.4% of the FG explants showed cell migration from the marginal zone, this applied to 93.3% of the VC cultures and 87.7% of the SGE cultures. At the start of the 2d week of cultivation, axonal fasciculation became more marked (Figure 1б), there was continued migration of glial and granule cells from marginal zones of the explants. In the explant growth zones we often observed formation of small re-aggregates consisting mainly of small, round cells which we identified as granule cells according to their morphological parameters. Some of the explants were flattened out and cleared; in the unspread explants there were a few clearing zones. The explants revealed an external granular layer consisting of granule cells, as well as a layer of Purkinje cells arranged in several rows (Figure 2a and б), which reproduces the histo-typical structure of the embryonic cerebellum. Formation of axonal myelin sheaths began on the 12th day of cultivation, and by the end of the cultivation period (21st day)

a significant part of the fibers of central and peripheral zones of the explants was myelinated in both FG cultures, and in the VC and SGE groups (Figure 2в).

Thus, intravital light microscopy observation of the dynamics of cytogenesis and histogenesis of explants of rat embryo cerebellum revealed, for all the above parameters, virtually identical morphogenesis of the cerebellum in the experimental and control series of cultures.

Light microscopy of semi-thin sections distinctly revealed organotypical architectonics of the cortex and subcortical parts of cerebellar explants. The marginal zones of cortical regions of the explants are formed by an accumulation of round bodies of granule and glial cells, which make up the external granular layer (Figure 3a). Deeper parts of the cortical plate of the explants contain the bodies of large neurons (Purkinje, Golgi cells; Figure 3a and б). The internal granula layer, which is inherent in cultures of neonate rate cerebellum was not pronounced in cerebellar cultures from 18-day embryos. Typically, there were individual or grouped large neurons in the subcortical regions of cerebellar explants formed mainly of a dense plexus of myelinated and unmyelinated fibers.

The cellular architectonics described above, in our analysis of semi-thin sections, were demonstrable in most FG, VC and SGE explants, and were consistent with findings obtained from intravital investigation of cellular organization of cultures, whereas in the other explants there were changes in spatial relations and dimensions of the external granular layer and layer of Purkinje cells. These changes were inherent in markedly thinned down explants in all examined series of cultures.

Thus, the histotypical organization and cyological features of neurons and glial cells in explants of PG, VC and SGE rat embryo cerebellum are indicative of progressive differentiation of these cells during cultivation and absence of pathological changes in them.

A comparison of findings of intravital microscopy of developing explants of cerebellum from 18-day FG, VC and SGE rat embryos to the results of light microscopy of semi-thin sections revealed that cellular differentiation and histogenesis of cerebellar structures were analogous in all three groups. A comparison of the results of the Purkinje-2 experiment to the results of studies of cytogenesis and histogenesis of the neonate rate cerebellum in orghanotypical cultures [3, 4, 6, 7] reveals some differences in time of formation and cellular organization of cerebellar explants. For example, in the cultures we studied, growth of nerve fibers from explants began 1.5–3 days later in all 3 groups, which can be attributed to a longer period of adaptation to cultivation conditions in the plastic containers and subsequent transfer of cultures into Maximov chambers. The other time parameters of explant development in the Purkinje-2 experiment were virtually the same as those described previously [3, 4, 6, 7]. Underdevelopment of the internal granular layer is among the morphological distinctions of explants we examined, and it is related to the fact that migration of granule cells forming this layer had not yet occurred in the organotypical cultures of the cerebellum of 18-day rat embryos. The changes we demonstrated in morphology and dynamics of formation were inherent in explants of all three experimental series, and they are apparently unrelated to the effect of weightlessness. These changes are most probably attributable to the specifics of cultivation conditions and the fact that, in these studies, we used the cerebellum removed at early stages of embryonic development. The findings warrant the conclusion that the effect of spaceflight factors on

morphogenesis of the cerebellum in the period from the 13th to 18th days of embryogenesis is not associated with cytopathological changes in differentiating neurons and glial cells of this structure that would lead to subsequent disturbances in cerebellar development in an organotypical culture for 21 days. At the same time, we cannot rule out the possibility that the changes in embryonic development of cerebellar structures that arise as a result of weightlessness are either not manifested in an organotypical culture or are compensated by virtue of the morphogenetic elasticity inherent in these cultures.

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NONINVASIVE EXAMINATION OF BONES DURING LONG-TERM HYPOKINESIA

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 22, No 1, Jan–Feb 88 (manuscript received 16 May 87) pp 30–33

[Article by V. S. Oganov, A. S. Rakhmanov, B. V. Morukov, Kh. A. Yanson, A. M. Tatarinov, V. Ye. Zaychik, S. K. Ternovoy, and C. Cann (USSR, United States)]

[English abstract from source] The effect of 120-day bed rest on skeletal bones of 25 volunteers was investigated by noninvasive methods, viz. gamma-photon absorption, ultrasonic and neutron-activation analysis. The test subjects were divided into 4 groups one of which served as control and three others used different countermeasures (drugs, exercise or drugs in combination with exercise). Calcium loss in skeletal bones was not more than 0.5% per month; calcium loss in leg tubular bones was 1 to 2% per month in 6 test subjects; calcium loss in heel bones was on the average 3–4% per month in the control, exercise and combination groups. No strict correlation between the negative balance of calcium and mineral content in leg compact bones and foot spongy bones was found. There was a correlation between changes in the mineral content of leg bones and ultrasound propagation along certain compartments of the tibial median surface. In terms of negative and positive trends leg and foot bones were in better condition in the drug group. The techniques used were assessed with respect to their diagnostic and prognostic value.

[Text] One of the importent problems of biomedical support of long-term spaceflights is still to prevent possible decrease in mechanical strength of the skeleton in view of bone demineralization and negative calcium balance observed in cosmonauts [1, 2, 6, 11, 18, 22]. It is difficult to solve this problem because of the lack of reliable and relatively simple means of quantitative evaluation of mineralization of bone, which generally is correlated with the mechanical properties of bones. However, mineralization as a function of strength is also far from unambiguous due to the distinct heterogeneity of these characteristics in bone tissue, insufficient investigation and complexity of actual metabolic processes associated with regeneration of bone and its adaptation to functional (mechanical) loads [6, 10].

Our main objective was to make a comparative evaluation of demineralization in different parts of the skeleton during long-term hypokinesia, as well as to assess the

efficacy of experimental means of preventing adverse changes in bones. In addition, we planned to compare the results of densitometric and ultrasonic tests.

Methods

Quantitative computerized tomography [13] was used to assess mineralization of spongiosa of lumbar vertebrae. Bone mineral content (BMC) was determined by gamma-photon absorptiometry [17] on the boundary between the median and distal thirds of the tibial and fibular dipahysis, as well as at 25% of the length of the radius and ulna (measured from the styloid process). Analysis of calcium content (CC) in the foot was based on prior local irradiation of the subject's food with neurons, followed by gamma-spectrometry [4]. Investigation of acoustic parameters of bone tissue was made using piezoelectric exponential waveguides firmly secured on a 10-mm base with standard ultrasonic pulsed equipment at a frequency of 120 kHz [3]. Measurements were taken along the middle of the medial aspect of the right tibia in 10 zones. The following parameters were analyzed on the basis of the results of ultrasonic measures: C_{10}—mean velocity of ultrasound in the 10 zones of bone length; ΔC—difference between maximum and minimum velocity of ultrasound over the zones in a distal direction [12].

We examined 4 groups of subjects: the 1st (control) consisted of 3 people who were submitted to hypokinesia (bedrest); 2d—4 people who took medication; 3d—4 subjects who exercised; 4th—4 people who combined exercise with pharmaceutical agents.

The pharmaceutical agents (xydiphone, tocopherol, glucamak, solizim, F-99) were given to the subjects following a protocol developed by A. I. Grigoryev and B. V. Morukov. Exercises were performed according to a program developed by I. B. Kozlovskaya and A. V. Ovsyannikov. We used four modes of exercise for muscles of the leg, thigh, back and neck: those involving speed, speed and force, force and passive extension of antigravity muscle groups. Cycle ergometers, isokinetic equipment and expanders were used for the exercises.

Results and Discussion

According to the results of computerized tomography, none of the experimental groups presented reliable decline in mineralization (MN) of lumbar vertebrae. Furthermore, mean MN exceeded the baseline by 12% in the control group (see Table).

Analysis of the results of gamma-photon absorptiometry revealed reliable decline of BMC in leg bones by the 120th day of hypokinesia—by 4.2–5.4% in 5 subjects of the 2d–4th groups and by 8% in 1 subject of the 1st group. Mean group BMC in leg bones virtually failed to differ from the baseline following bedrest. We could merely detect a tendency toward decline of BMC in the 1st and 3d groups of subjects and increase in this parameter in the 4th group. In the latter group, a reliable increase in BMC of arm bones (ulna and radius) was noted after 120 days of hypokinesia.

For qualitative evaluation of preventive measures, the results of gamma-photon absorptiometry were expressed as follows: 0—BMC change within the method's margin of error, "+" or "−" —increase or decrease, respectively, exceeding the margin of error of the method. After addition of rank evaluations for months in each group, we found that the groups are arranged in the following order according to decline of the effect of normalization of mineral metabolism: 4th, 2d, 1st, 3d.

Changes in mean group parameters of condition of bone (% of baseline values) and calcium balance after 120-day hypokinesia

Group	Arm bones	Leg bones			Lumbar vertebrae	Foot bones	Calcium balance
	BMC	BMC	C_{10}	ΔC	MN	CC	
1	−1,3±1,6	−2,6±2,9	−2±0,7	−16±4*	+12,6±7,6*	−15±1,5*	−24,3±6,2*
2	+1,1±1,7	−0,7±1,8	0	−14±4*	−0,8±2,6	+3,2±2,3	−5,2±4,7
3	+3,9±2,4	−4,1±1,0	0	−30±6*	+4,2±2,3	−16,3±0,4*	−11,5±1,6*
4	+12,5±2,6*	+4,2±1,7	0	−15±5*	+2,3±5,3	−12,3±0,8*	−10,6±3,0*

* $p<0,05$

Neutron-activation analysis revealed that 120-day bedrest leads to significant decrease in calcium content of the foot. CC of the foot diminished to the same extent in the 3d group as the 1st.

On the whole, the reuslts of neutron-activation analysis coincided with conclusions derived from gamma-photon absorptiometry to the effect that mineral metabolism was better in subjects of the groups were pharmaceutical agents were used for preventive purposes. This conclusion coincides with the results of calcium balance studies.

Investigation of individual acoustic characteristics of bones during bedrest revealed a reliable declin of C_{10} in 8 out of 15 subjects (2 from each group). Individual values of ΔC diminished during bedrest in 2 subjects of the 1st group, 3 of the 3d, 2 of the 4th and 1 of the 2d group.

As can be seen in the table, after hypokinesia mean group C_{10} for the tibia did not differ from baseline values. Mean group ΔC, which characterizes adaptation properties of bone to mechanical loads, diminished reliably by the 120th day of bedrest in the 3d group of subjects. We used the above-described ranking method to make a general evaluation of differences between group parameters. The groups were arranged in the following order, according to decline of effect of normalization of acoustic properties of bone: 2d, 4th, 3d, 1st.

Thus, there was good coincidence of results of ultrasonic tests and gamma-photon absorptiometry, the main conclusion being that the condition of leg bones during bedrest was better in individuals who used pharmaceutical agents for preventive purposes (2d and 4th groups) than in others.

Summarizing the results of long-term studies using bedrest, which had been conducted in the last 15 years in the Soviet Union and abroad, it can be concluded that calcium loss constitutes about 0.5% per month (according to results of balance studies), MN decrease in spongy structures of the skeleton constitutes 1–5% per month for the calcaneus. No reliable decline of minerals in long bones of the arm was demonstrated [7–9, 15, 23].

A comparison of these data to existing conceptions of distribution of minerals in the human skeleton [16] leads us to concur with the opinion [22] that most minerals apparently exit from the parts of the skeleton in which intravital measurement is difficult (for example, epiphyseal and metaphyseal regions of long bones, iliac bone,

etc.). The results of model experiments with primates [21] and clinical observations are indicative of the need to consider the condition of compact bone, in particular, when the skeleton does not carry a static load [19]. This is also indicated by the results of analytical calculations which took into consideration the correlation between rate of renewal of compact and spongy bones [24], their weight [16] and mineralization [5].

The reuslts of these studies confirm the fact that the mean monthly rate of excessive loss of calcium by man during bedrest without intervention ("pure" hypokinesia) constitutes about 0.5% of total content in the body. Preventive measures (exercise, drugs) reduce this loss by more than one-half.

Different methods of noninvasive demonstration of minerals in different parts of the skeleton are used not only for diagnostic purposes, but for determination of the source or sources of the above-mentioned "escape" of calcium from the human body during bedrest.

The results of gamma-photon absorptiometry are indicative of insignificant decrease in mineral content of long bones of the leg, even during "pure" hypokinesia (1st group). Only one of the subjects showed a mean monthly 2% loss of minerals, whereas mineral content did not change in all the rest. In other groups using some form of preventive measures, the rate of decline in mineral content reached 1.4% per month in some subjects. It is known that compact bone makes up about 80% of the skeleton (75% for leg bones) [16], and its mineralization is considerably higher than that or trabecular bone. Consequently, even with a slow rate of demineralization (1–2% per month), loss of minerals could be significant here. At the same time, we failed to demonstrate a correlation between results of gamma-photon absorptiometry and calcium balance studies.

As for the condition of spongiosa, the results of computerized tomography do not agree with the existing conception of rapid rate of osteoporosis of the spine (5–7% per month) during hypokinesia or weightlessness. Demineralization of lumbar vertebrae demonstrated previously in an analogous experiment was also less marked than the estimated levels [14]. It can be assumed that, in healthy subjects submitted to hypokinesia (unlike immobilized patients), functional activity of the vertebral system of ligaments and muscles [20] is sufficient to retain the baseline level of bone MN for 4 months.

The results of neutron-activation analysis of CC in foot bones are similar to those of balance studies. However, here too no reliable correlations were found. In addition, it should be noted that, even with consideration of high lability of Ca metabolism in the calcaneus, its absolute loss is minimal and prognostically insignificant. Differences in severity of reactions of spongiosa of the calcaneus and spine had already been noted in analogous studies [14]. From the standpoint of the diagnostic purposes of this study, it can be assumed that, considering the conservative nature of changes in compact bone structures, gamma-photon absorptiometry of long bones may turn out to be more informative for long-term (over 4 months) studies. In the same diagnostic respect, demonstration of a reliable correlation ($r=0.68$) between changes in BMC and mean speed of ultrasonic testing may be considered a positive result.

On the whole, in our opinion the effectiveness of combining gamma-photon absorption and ultrasonic diagnostic methods is attributable to the high sensitivity of acoustic

characteristics of bones to change in their biomechanical status and the high precision of assaying minerals.

Thus, the results of this investigation basically coincide with the findings of other studies [7, 8, 9, 15, 23], but unlike the latter, we have shown that the chosen set of pharmaceutical agents neutralizes skeletal demineralization well. This conclusion is confirmed by the results of noninvasive methods of measuring minerals in different parts of the skeleton by means of gamma-photon absorption in long bones of the leg and neutron-activation analysis in bones of the foot. At the same time, we did not obtain any statistically reliable data that would enable us to identify the principal sources of calcium loss under conditions simulating a decrease in gravity load on the skeleton.

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612.014.477.064]015.4:612.73].076.9

EFFECT OF DIFFERENT DOSES OF α-HYDROXYDIMETHYL-γ-AMINOPROPYLIDENE BISPHOSPHONATE ON RAT BONES

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 22, No 1, Jan–Feb 88 (manuscript received 12 May 87) pp 34–37

[Article by V. N. Shvets]

[English abstract from source] For 10 days rats were subcutaneously injected with alpha-hydroxydimethyl-gamma-aminopropylidene biphosphonate in the dose range 0.005 to 5 mg/kg/day. As shown morphometrically, the mass of spongy bone increased linearly increase with the dose. It was found that the drug affected primarily the highly metabolic component of spongy bone. The drug had a systemic osteotropic effect and modified the number of osteocytes significantly. When the drug was injected for a long time (up to 60 days), the number of osteoclasts decreased and the proportion of cells containing more than one nucleus remained within normal limits. The number of osteoblasts either diminished (in long bones) or remained unchanged (in torso and pelvic bones). It is concluded that the osteotrophic effect of the drug is mediated via its action on bone resorption the rate of which is inhibited; this is responsible for bone mass growth.

[Text] Choice of agents for the treatment and prevention of osteoporosis in weightlessness is one of the important problems of space medicine. Bisphosphonates are among such agents. The biological action of xydiphone (hydroxyethylidene bisphosphonic acid—HEBP) has already been well-studied. On the basis of numerous experimental and clinical observations [8], it is believed that xydiphone has osteotropic action, but only in rather large doses, and this is often associated with transient side-effects in the form of osteomalacia and formation of fibrous bone marrow. This circumstance limits, to some extent, the use of xydiphone in clinical practice. A comparison of the effects of various bisphophonates revealed that the effect of such compounds depends on their structure [11]. In recent years, several hydroxyalkylidene bisphosphonic acids have been synthesized that contain an amino group [1]. The high solubility of such compounds at any pH is a valuable quality with regard to their use for disturbances referable to calcium metabolism. Such compounds include α-hydroxydimethyl-γ-aminopropylidene bisphosphonate (APB). Thus far, the biological action of the only representative of this class, α-hydroxy-γ-aminopropylidene bisphosphonate, which is similar in structure to APB, has been

described in the literature [8, 11]. Does APB have osteotropic action? Exploration of this question was the purpose of our investigation.

Methods

Hypodermic injections of APB, in the form of monopotassium salt (pH 7.4), in doses of 0.005, 0.01, 0.1, 1 and 5 mg/kg/day for 10 days, were given to male Wistar rats weighing 300 g. The tibia, vertebra (from the lumbar spine), sternum and ilium were extracted from decapited animals (10 per group) 24 h after the last injection. Animals in another group (16 rats) were given APB per os in a dosage of 0.5 mg/kg/day for 45 days. At the end of this experiment, we extracted the ilium from which impressions were prepared (8 per slide, 3 slides per rat) of the crest region in cross section by the Addison method [3]. Osteoclasts containing different numbers of nuclei (2 or more) were demonstrated in the impressions using an enzyme tracer (reaction to β-hydroxybutyrate dehydrogenase). Bones of all experimental rats were fixed in 10% calcium formol, decalcified in 6% trichloroacetic acid and imbedded in paraffin. Longitudinal sections (5-7 μ) were prepared through the middle of the bone, stained with hematoxylin and eosin or toluidine blue. The following parameters were identified by the morphometric method using an ocular grid or ocular micrometer: volume density of the entire spongiosa represented in the tibial metaphysis, vertebral body, sternum and ilium; volume density of spongy bone localized in the zone of the primary spongiosa (lens 40×, eyepiece 7×) of all above-mentioned bones; width of epiphyseal growth plate (EGP) and its cartilaginous zones; number of osteoblasts and osteoclasts (per 5 fields) in the region of primary spongiosa (lens 40×, eyepiece 10×). Calculation of cells was made per field of vision (0.36 mm^2), and that of volume density of bone was made as previously described [2]. All of the digital data were processed according to Student.

Results and Discussion

Figure 1 illustrates the results of measuring volume density (mass) of spongy bone in different parts of the skeleton. It is easy to see that, with increase in dosage of the agent, there was linear increase in mass of all spongy bone only in bones of the extremities (Figure 1), whereas this parameter did not change in bones of the trunk and pelvis. These findings warrant the belief that APB appears to have local action on expressly those parts of the skeleton that carry the greatest mechanical load. In this respect, the bones of trunk differ, as we know, from those of the limbs in that they have less functional activity. According to our obsservations, some segments of spongy bone located within either a single bone or the entire skeleton differ in rats in extent of reaction to such factors as immobilization, weightlessness and hypergravity. The first to react are the segments of bone in which metabolic rate is higher. Considering this feature, it became necessary to assess the effect of APB on the part of the spongiosa with rapid metabolism. Such bone is situated in the region of primary spongiosa just under the cartilaginous growth plate [10]. If APB has systemic osteotropic action, we should expect changes in mass of spongy bone in the primary spongiosa zone of different bones. Conversely, absence of any effect would confirm the localized nature of APB action, extendng only to extremital bones.

Figure 1 shows that the mass of bone tissue in the region of primary spongiosa increased linearly with increase in dosage of APB in all bones, with the exception of the sternum. Bone mass reached a plateau in bones of the limbs and pelvis starting with a dosage of 0.1 mg/kg, i.e., there was a saturation effect that was absent in, for example, the spine (see Figure 1).

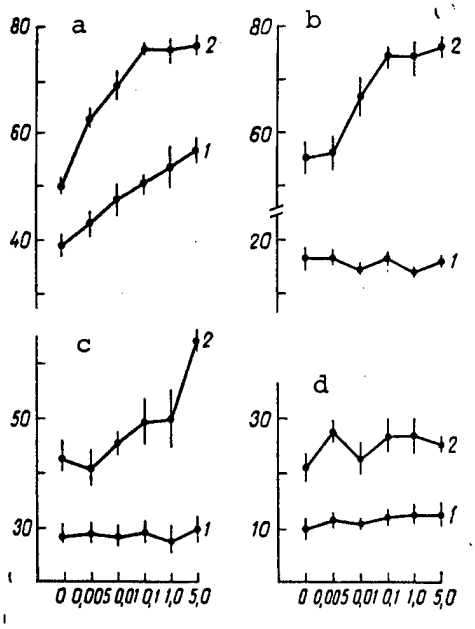

Figure 1.
Volume density of spongiosa in tibia (a), ilium (b), vertebra (c) and sternum (d)
X-axis—APB dose (mg/kg), y-axis—bone mass (% of total volume of medullary canal, $M \pm m$)
1) mass of entire spongy bone
2) mass of spongy bone localized in primary spongiosa region

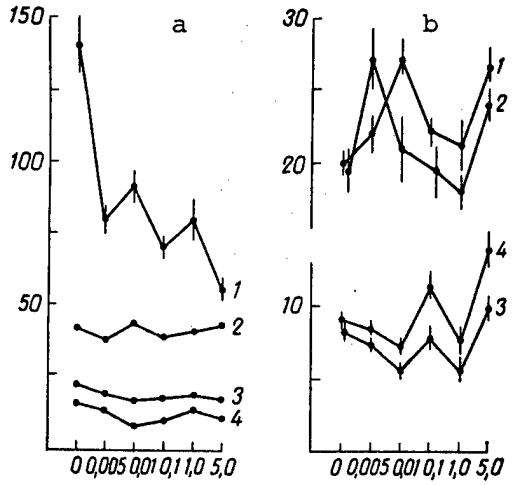

Figure 2.
Number of osteoblasts (a) and osteoclasts (b) in primary spongiosa zone of tibia (1), ilium (2), sternum (3) and vertebra (4)
X-axis—APB dose (mg/kg); y-axis—number of cells per 0.36 mm^2 ($M \pm m$)

Thus, the results of this study revealed that APB has systemic osteotropic action and that it affects primarily the bone region with high metabolic activity. This conclusion was derived from a comprehensive study of different regions, whereas upon measurement of the entire mass of spongy bone in long bone metaphyses, in the entire veretebral body, sternum and ilium we failed to demonstrate such a pattern (see Figure 1). The difference in informativeness of these two parameters (volume of entire spongy bone and volume of its highly metabolic part) may be related to the fact that the share of bone in the region of the primary spongiosa is only a small part of its total mass. Indeed, measurements revealed that primary spongiosa constitutes 25–30% of total volume of spongy bone in the tibial metaphysis and only 10–15% in bones of the trunk and pelvis. For this reason, a linear increase in overall bone mass was observed only in the tibia. The sternum occupies a special place in this regard, and its spongy bone showed no response to APB. It may be that this is attributable to the fact that the metabolic rate of spongy bone in the sternum is very slow, as compared to other bones, and for this reason there is small difference between primary and overall spongiosa, i.e., the sternum is relative more inert than all other tested bones.

Some amount of spongiosa is directly related to EGP function, provided the bone grows in length. The width and structure of EPG in all tested bones remained at the control level, and was unrelated to concentration of APB (see Table), i.e., the agent did not change bone growth for at least 10 days. Evidently, this is attributable to functional impairment of bone cells that determine bone reactions. Figure 2 shows that the number of osteoblasts in the tibia decreased progressively with increase in dosage of APB, but in the other bones the number of these cells remained at the

EGP width (μ) in different skeletal bones with use of APB ($M \pm m$)

APB dose, mg/kg	Skeletal bone			
	tibia	verteb.	sternum	ilium
0,005	250±6	103±4	120±2	169±9
0,01	244±6	105±4	125±6	172±9
0,1	238±6	96±5	118±3	180±9
1,0	245±8	107±4	118±4	178±14
5,0	238±7	103±3	125±3	172±3
H_2O	252±10	95±3	123±4	170±6
0	254±11	103±4	118±5	172±7

control level (see Figure 2a). The absence of correlation between change in bone mass and number of osteoblasts indicates that accumulation of bone tissue is not attributable to osteoblast hyperfunction, but apparently to inhibition of the process of bone resorption. Examination of the population of osteoclasts revealed that their number did not diminish, but it was subject to fluctuations, or else it changed within the range of the physiological norm under the influence of different doses of APB (Figure 2b). Such reaction by the osteoclast population should have been indicative of increase or, at least, retention of the rate of bone resorption. If this were so, there would be no accumulation of bone mass. Consequently, this effect cannot be attributed solely to quantitative data, but it must be viewed in direct relation to bone cell function. There are a number of data proving the cellular mechanism of action of some bisphosphonates [4–9] that alter the function of different types of cells. It has been shown that bisphosphonates have a similar effect on the osteoclast population: the number of the latter increases with concurrent decrease in their functional activity [4, 8]. With respect to APB we do not have such data, but the possibility cannot be ruled out that osteoclast function also diminishes under the effect of this bisphosphonate. The fluctuation and asynchronous nature of osteoclast response illustrated in Figure 2 in different bones is unrelated to APB dosage, which is indicative of destabilization of histogenesis of these cells. One would think that with longer administration of APB there will be a more distinct reaction by the osteoclast population, and then we shall be able to determine how APB affects osteoclasts. Investigation of this matter revealed that long-term (up to 45 days) administration of APB to intact rats was associated with decrease in number of osteoclasts: from 430 ± 8.7 in the control to 113 ± 4.5 under the effect of APB. There was the same correlation between osteoclasts containing a different number of nuclei as in the control. This proves that no intrapopulation changes occur under the effect of APB, and it most likely affects osteoclast histogenesis, apparently on the level of either hemopoietic stem cells that originate precursor cells, or on the level of the latter, as a result of which replenishment of the pool of highly differentiated osteoclasts (morphologically identified) is inhibited. Thus, the increase in bone mass is directly related to decrease in number of mature osteoclasts and (apparently) their function, which is discretely manifested already within the first 10 days of APB action, which causes accumulation of bone mass.

Thus, APB has an effect on the part of spongy bone with high metabolic rate, the mass of which increases as a function of dosage of APB. This effect is due to development of an imbalance between bone synthesis and resorption. The latter process prevails over the former, and it is the dominant factor determining the osteotropic effect of APB. We cannot fail to stress the fact that APB is a highly effective agent, as compared to other bisphosphonates (for example, NEBP).

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ROLE OF OPIOID PEPTIDES IN PATHOGENESIS OF VESTIBULOVEGETATIVE DISORDERS

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 22, No 1, Jan–Feb 88 (manuscript received 22 Jan 87) pp 37–40

[Article by V. S. Shashkov, Yu. V. Drozd, V. V. Yasnetsov, Ye. Yu. Galkina and Yu. I. Ryumin]

[English abstract from source] The study was carried out using 12 noninbred male cats and 14 white rats. In response to vestibulo-autonomic disorders the rats showed a decrease of beta-endorphin in the midbrain, medulla oblongata and hypothalamus as well as a reduction of met-enkephaline in the hypothalamus and medulla oblongata. The concentration of met-enkephaline in the adrenals increased and that of beta-endorphine in blood did not change. This may be attributed to the intraneuronal redistribution of opioids and their transfer to the pituitary or release into the cerebrospinal fluid. Opioid variations give evidence that vestibuloautonomic disorders in rats do not stimulate the pituitary-adrenal system. The cats were exposed to vestibulo-autonomic disorders and subsequent intracerebroventricular administration of regulatory peptides or injection of opiate receptor blockers into the chemoreceptor trigger zone. It was demonstrated that naloxone, gamma-endorphine and des-Tyr-gamma-endorphine were effective in protecting the vestibular function whereas ICI 154, 129 (a selective antagonist of delta-receptors) was practically ineffective.

[Text] At the present time, the search is continuing in our country and abroad for new effective pharmacological agents for the prevention and treatment of vestibulovegetative disorders (VVD). The difficulty in developing new preventive and therapeutic agents is related to the lack of an acceptable theory of pathogenetic bases of VVR. It is known that such an unpleasant symptom as vomiting may arise either as a reflex without immediate precursors [13] or as a result of neurohumoral and hormonal changes [1, 5, 6]. In 1983, G. H. Crampton [10] assumed that there are hypothetical VVD substances in spinal fluid, which appear in the 3d ventricle of the brain during motion and then pass into the 4th ventricle where, by acting on a chemoreceptor trigger zone (CTZ), they elicit vomiting and other manifestations of VVD, such as pallor, perspiration, impairment of gastrointestinal peristalsis, etc. The opioid peptide, met-enkephalin, may be one of these substances. However, in the opinion of H. S. Borison, CTZ of the 4th ventricle is not involved in the pathogenesis

of VVD and it is not expedient to search for pharmacological blockers of CTZ vomiting receptors for the prevention of vomiting in the presence of VVD [8]. Our objective here was to investigate the opioid peptides contained in the animal brain during simulation of VVD and to examine, from this vantage point, the role of CTZ in its pathogenesis, as well as to test new pharmacological agents for the prevention and treatment of VVD.

Methods

This study was conducted on 12 male mongrel cats and 14 mongrel white rats. In the 1st series of experiments, a cannula was implanted in the 4th ventricle of the brain in accordance with atlast coordinates [15] P=11, H= 4.5 and L=0 in anesthetized (pentobarbital sodium, 30-40 mg/kg, intraperitoneally) cats. The position of the cannula was subsequently verified using Evans' dye upon dissection of the animals (nembutal in a dosage of 60 mg/kg was used for ethanasia). Scopolamine (an M-choline blocker), β-lipotropin (precursor of opioid peptides), γ-endorphin and des-tyr-γ-endorphin, in doses of 0.1–100 μg, were dissolved in sterile isotonic solution of sodium chloride and injected into the 4th ventricle in a volume of 50 $\mu\ell$ with a microsyringe. Naloxone (specific antagonist of opiate receptors), ICI 154, 129 (selective antagonist of δ-opiate receptors) were injected into the 4th ventricle in doses of 5–100 μg in the same volume.[1] Immediately after implanting the cannula we tested the effect of the agents administered intraventricularly on amplitude, duration and latency period of evoked potentials of the cortical somatosensory zone of the contralateral hemisphere in response to stimulation of the ulnar nerve with square-wave pulses at a frequency of 0.24 Hz, 0.4 ms long with 7 V amplitude. Evoked potentials were recorded on a Nihon Kohden (Japan) electrophysoilogical unit. The animals were submitted to motion by the method of L. A. Radkevich [2]. Degree of motion sickness was graded on the scale proposed by K. B. Suri [17]. Just prior to rocking the animals, the tested agents were injected to protect the vestibular system.

In the 2d series of experiments, which were performed on rats, we assayed opioid peptides—β-endorphin and met-enkephalin—in brain structures, blood and adrenals after rocking the animals for 1 h by the above-mentioned method. The animals were decapitated using a guillotine, their brain was extracted, it was washed in 50 μM HEPES solution and frozen to —70°C for storage. Samples of brain structures were weighed and homogenized. Liquid high-pressure chromatography was used for separation of peptides and radioreceptor analysis, for assaying peptide concentrations.

Results and Discussion

After intraventricular (I.V.) injection of morphine in a dosage of 10—100 μg, we observed growth in amplitude of evoked potentials with a maximum after 3—4 min; 7—15 min after injection, the amplitude of evoked potential (EP) returned to the baseline. EP amplitude did not change appreciably when naloxone in a dosage of 10—20 μg was injected 1—2 min before morphine.

There was also no appreciable change in EP amplitude after injection of leu-enkephalin in a dosage of 100 μg. Interestingly enough, we demonstrated postural instability and

[1]The authors wish to thank Yu. A. Pankov, corresponding member of the USSR Academy of Medical Sciences, Professor M. I. Titov and O. S. Medvedev, doctor of medical sciences, for being kind enough to furnish these products.

rocking, in addition to vomiting, when morphine was given I.V. to waking animals whose behavior was unrestricted. We also observed vomiting after injection of leu-enkephalin in a dosage of 10—100 μg; however, there was considerably less marked impairment of coordination of movements, or else it did not appear at all.

Table 1. Preventive effect of drugs with simulation of VVD in cats

Parameter	Placebo	Scopolamine		ICI 154,129	β-Lipotropin	Naloxone	γ-Endorphin	Des-tyr-γ endorphin
		I.V.	s.c.	I.V.	I.V.	I.V.	I.V.	I.V.
Dosage, μg	Saline 50	20—30	0,1—10	20—100	10—100	5—10	10—100	10—100
Severity of VVD, score	12,1±0,5	4,3±1,9*	11,7±2,3	8,5±2,5	10,5±1,4	6,0±2,4*	5,4±2,3*	6,6±1,6*

*$p<0.05$ (Student's criterion).

Table 2. Opioid peptide content of rat brain structures, blood and adrenals

Concentration pmol/ℓ	Number of cases	Hypothalam.	Midbrain	Medulla oblong.	Hypophysis	Adrenals	Blood
Met-enkephalin	Experiment n=8	910±107	240±42	190±30*	1250±130**	73,0±4,6**	—
	Control n=6	1110±164	190±28	290±24	760±90	48,1±5,8	—
β-Endorphin	Experiment n=8	156±44	17±5	12±2	11880±890**	—	0,165±0,031
	Control	197±80	21±3	18±4	7670±1360	—	0,142±0,024

*$p<0.05$.
**$p<0.01$ (Student's criterion).

It may be that onset of sensory motor conflict with administration of opioids is elicited through CTZ μ-receptors, the main agonist of which is morphine. We failed to demonstrate an appreciable effect of the above agents on duration of latency period or duration of the entire EP complex.

Table 1 lists data concerning the preventive effect of some peptides and blockers of opiate receptors after I.V. injection, as compared to the most effective (against VVD) pharmacological agents, scopolamine when given I.V. and hypodermically (s.c.). As can be seen in Table 1, γ-endorphin (a peptide with neuroleptogenic activity and mild opiate properties), des-tyr-γ-endorphin (synthetic analogue of γ-endorphin, which does not interact with opiate receptors), naloxone (blocker of μ- and δ-opiate receptors) and, to a lesser extent, ICI 154, 129 (selective δ-receptor blocker) have a preventive effect that is comparable to that of scopolamine s.c. When given I.V., scopolamine does not have vestibuloprotective action. Thus, by affecting the animals' CTZ one can enhance their vestibular resistance. The mechanism of protective action is related to blocking μ- and δ-opioid receptors. We cannot rule out the possibility that the mechanism of the protective action of γ-endorphin, a peptide that has minimal

interaction with opiate receptors, is related to activation of peptidases, which are enzymes that destroy opioid peptides. The mechanism of protective action of des-tyr-γ-endorphin is perhaps effected through blocking of dopamine receptors [3].

Table 2 lists data concerning opioid peptide (β-endorphin and met-enkephalin) content of rat brain structures, blood and adrenals. As can be seen in this table, after rocking there was a tendency toward increase in opioid content of the hypophysis ($p<0.05$), decrease in β-endorphin in the medulla oblongata, midbrain and hypothalamus, and decrease in met-enkephalin in the hypothalamus and medulla oblongata ($p<0.05$). This could be related to intraneuronal redistribution of opioids [4]. However, it is more likely that, in this case, they pass into spinal fluid [3, 10]. It is interesting to note that the increase in β-endorphin content of the hypophysis was not associated with a statistically significant increase in its concentration in blood. In man, on the contrary, with development of VVD symptoms there is manifold increase in blood β-endorphin content [5]. The increase in rat adrenal met-enkephalin content ($p<0.05$) is apparently indicative of absence of their activation when VVD are simulated [4]. All of the foregoing indicates that development of VVD in rats is not associated with activation of the pituitary-adrenal system.

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UDC: 613.693:[612.766.1-019:599.82

MACACA RHESUS TOLERANCE TO +Gz ACCELERATIONS

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 22, No 1, Jan–Feb 88 (manuscript received 24 Mar 87) pp 40–45

[Article by I. F. Vil-Vilyams, V. I. Korolkov, V. P. Krotov, A. A. Shipov, V. G. Andreyeva, L. A. Tabakova, S. F. Kholin, A. N. Truzhennikov and Yu. V. Gordeyev]

[English abstract from source] The procedure of Cosmos-1514 selection and training of rhesus-monkeys included +Gz acceleration tests. Two experimental series were performed. In the first experimental series (52 monkeys) acceleration tolerance was determined with respect to general health condition and behavioral responses of animals, electrocardiographic data (in three standard leads), heart rate and respiration rate. In the second experimental series acceleration tolerance was measured on the basis of blood pressure and flow velocity in the common carotid artery. Rhesus-monkeys exhibited noticeable individual variations in +Gz tolerance as well as in circulation responses to this exposure. The tests helped to select flight animals with a high level of acceleration tolerance.

[Text] It is known that the body's reaction to accelerations is determined by a number of factors, among which level of accelerations, duration of exposure to them, rate of their build-up, direction in relation to the torso and functional state of the body are of substantial significance [1].

Circulatory disturbances during exposure to accelerations are the most significant, as compared to other changes, and they hold a prominent place in the genesis of physiological reactions.

Bearing this in mind, in the course of selecting and preparing monkeys for a flight aboard Cosmos-1514 biosatellite, studies were pursued to assess their tolerance to long-term accelerations. Special attention was given to the circulatory system.

Methods

These studies were conducted on 57 male Macaca rhesus monkeys (3–4 years old) weighing 3.0–4.4 kg.

Waking animals were fixed in a standard chair without a protective cabin, which was placed on the arm of a centrifuge. The back of the chair was inclined at an angle of

15° in relation to the vector of accelerations. The animals were rotated in the dark to rule out optokinetic stimuli.

There were two rotation sessions, one to familiarize the animals with rotation and the main test.

In the first session, the animals were exposed to +Gz accelerations in the form of a 2–G plateau for 30 s. Acceleration and deceleartion rate constituted 0.1 G/s.

During the main test, the animals were exposed to +Gz accelerations which varied in time according to the protocols; 1) up to 5 G, and 2) up to 11 G.

There was an interval of at least 5 min between the preliminary and main rotation session.

All of the tests were divided into two series, depending on the set of physiological parameters recorded.

In the 1st series (52 monkeys), we determined tolerance to accelerations on the basis of analysis of changes in ECG parameters (in 3 standard leads), heart rate (HR) and respiration rate (RR).

In the 2d series (5 monkeys), we recorded additionally arterial pressure (BP) and linear blood flow velocity (LBF) in the common carotid to assess acceleration tolerance.

In all of the experiments, in assessing the monkeys' tolerance to accelertions, we took into consideration their general condition and behaviorala responses, as well as the physiological data obtained after stopping the centrifuge.

In the 2d series, 4-5 weeks before the experiments we implanted under nembutal anesthesia a plastic cuff, in which we installed a pressure tensometer (Konigsberg Instruments, Pasadena, USA) and LBF ultrasonic sensor, on the common carotid artery. The cuff and measuring equipment, which were developed and manufactured by L. & M. Electrons [sic] (USA), were kindly furnished by NASA's Ames Research Center.

The wiring from the sensors were under the skin of the back and they were removed 3–4 weeks after implantation of the cuff on the vessel. An animal was considered prepared for the experiment 1–2 weeks after this operation.

The pressure tensometer was calibrated before and after rotating the animals on the centrifuge by comparing readings to pressure in the femoral artery measured by the direct method using transcutaneous puncture. During calibration, BP was changed by intravenous injection of mezaton [neosynephrine] and nitroprusside.

Results and Discussion

In the 1st series of studies, tolerance to accelerations following protocol 1 was rated as being good in 11 out of 38 tested monkeys (29%) and low in 27 (71%).

Tolerance to accelerations in monkeys with good resistance constituted 5.0 G and in those with low resistance, 1.9 ± 0.1 G.

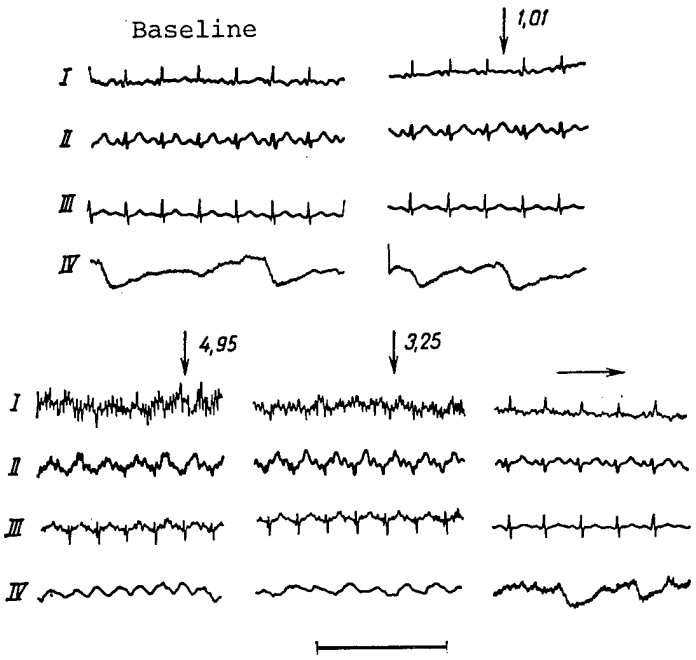

Figure 1. Typical example of ECG and respiratory changes in monkeys with good tolerance to +Gz accelerations in protocol 1

Here and in Figure 2:

I, II, III) first, second and third standard ECG leads, respectively

IV) pneumogram

Digits near vertical arrows refer to magnitude of accelerations (G); horizontal arrow indicates aftereffect period. Time scale—1 s

In animals with good tolerance, there were no signs of pathological ECG changes throughout the exposure period or in the aftereffect period (Figure 1). HR changes were characterized by sinus tachycardia (from 186 –252/min in the baseline period to 252–300) and moderate increase in RR (from 24–36 to 30–54/min).

In the aftereffect period, the general condition of this group of animals was good: active behavior, normal coloration of integument and visible mucosa, HR and RR reverted to baseline values within 4–5 min.

Serious disturbances of cardiac rhythm and conduction were noted in animals with diminished tolerance: sinoauricular and atrioventricular blocks, blockade of branches of His' bundle, migration of supraventricular pacemaker, polytopic and group extrasystoles, atrial flutter (Figure 2).

In the aftereffect period, all animals in this group presented with pallor of the integument and visible mucosa, sluggishness and inhibition of responses, which lasted over 15 min in a number of cases, slow (within 10–15 min) restoration of HR and RR to baseline values.

With protocol 2, tolerance to accelerations was good in 1 out of 14 monkeys (7%) and diminished in 13 (93%). In the monkey with good tolerance the level constituted 10.0 U and in those with diminished tolerance, 4.1 ± 0.9 G. On the whole, the animals

tolerated accelerations on the second protocol worse than on the first. Analysis of the ECG and general condition of the animals revealed changes in the same direction as those observed with use of protocol 1.

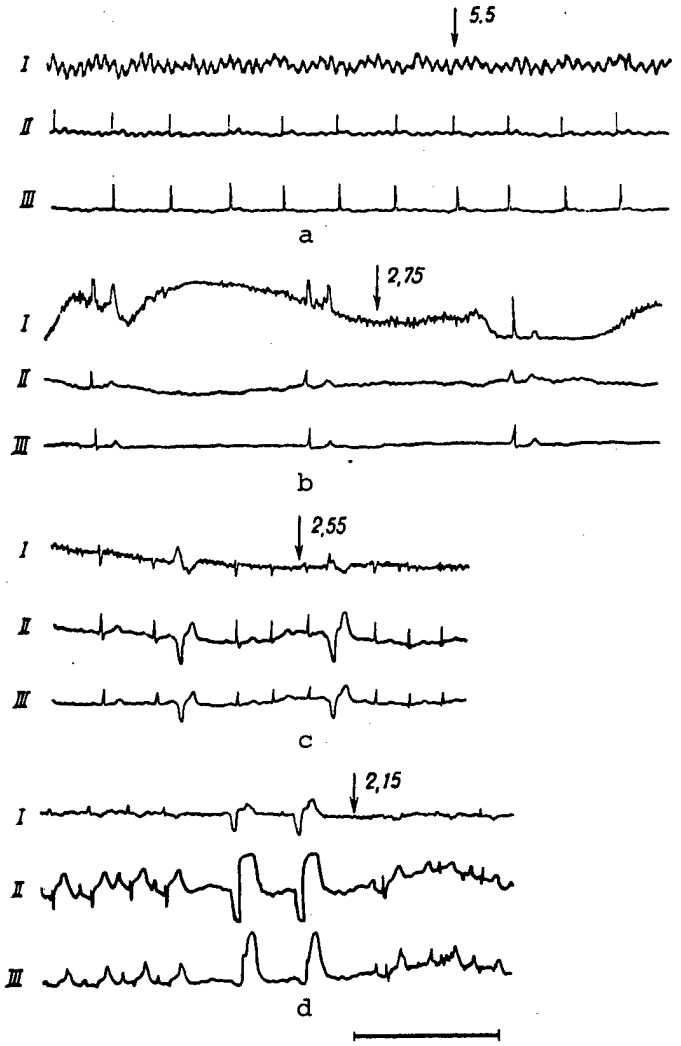

Figure 2. Examples of ECG changes in monkeys with diminished tolerance to +Gz accelerations using protocol 1
a) atrial flutter with 4:1 atrioventricular conduction
b) atrioventricular rhythm
c) isolated monotopic dextroventricular extrasystoles
d) atrioventricular block with substituted ventricular systoles
Vertical arrows pointing up indicate deceleration.
Other designations are the same as in Figure 1

In the 2d series, acceleration tolerance was diminished in all 5 monkeys with planted BP and LBF sensors when following protocol 1. Analysis of the data revealed that the response of the circulatory system to accelerations was highly individual in these animals.

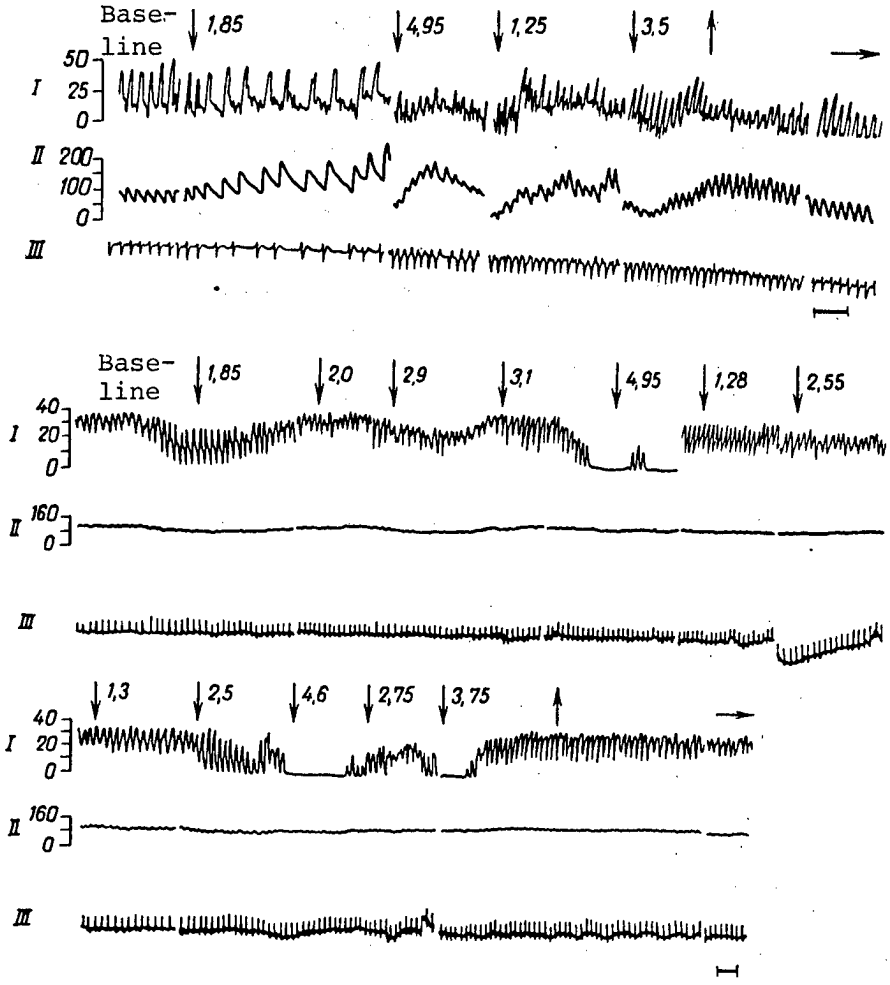

Figure 3. Examples of changes in hemodynamic parameters of monkeys with diminished tolerance to +Gz accelerations with protocol 1

a) the monkey, Tangir [Translator's note: the letters, a and b not shown
b) the monkey, Flint in source figure]

I, II, III) LBF (in cm/s), BP (mm Hg) and ECG, respectively

Other designations are the same as in Figures 1 and 2

Thus, with one type of response, we observed elevation of BP and slowing of carotid LBF to 50%, bradycardia followed by 40–50% increase in HR (Figure 3a), impairment of cardiac rhythm in the form of atrioventricular block with subsequent normalization.

With another type of response (Figure 3b), there was brief (for 5–6 s) decline of BP followed by hypertension. LBF slowed down and, at some stages of exposure to accelerations, particularly at the maximum, it dropped to 0 for 2–3 s. HR exceeded the baseline throughout the exposure period. Three episodes of relative bradycardia were observed with exposure to 2.7 G.

With the intermediate type of reaction, there were moderate changes in recorded hemo-

With the intermediate type of reaction, there were moderate changes in recorded hemodynamic parameters. There was a mild (10–15%) hypertensive response, with moderate (10%) increase in LBF. At the start of exposure HR diminished and at the end it increased (by 20-30). On the ECG, we observed a sinus rhythm with one episode of relative bradycardia at the start of deceleration of the centrifuge.

It is important to stress that pathological ECG changes developed in all tested animals at different BP and LBF levels. By the time the centrifuge was stopped, the recorded hemodynamic parameters virtually failed to differ from the baseline.

Tolerance to accelerations using protocol 2 was diminished in the 2d series of experiments. There were less marked individual differences in responses that with use of protocol 1. At the start of exposure to accelerations (up to 4 G), all tested animals showed a 30–50% elevation of BP. At peak accelerations, the hypertensive reaction became more marked in some monkeys, constituting 160–180% of the baseline, whereas in others it dropped to 40–60%. There were varying degrees of LBF slowing, to the extent of arrested blood flow in some animals already at 4 G. When accelerations increased to 4 G, HR usuall slowed down, after which bradycardia either persisted to the end of the exposure period, or else was followed by tachycardia. Impairment of cardiac rhythm in the form of a few episodes of atriventricular rhythm and dissociations appeared only during the period of centrifuge deceleration. By the time it was stopped, a sinus rhythm was observed in all animals. We failed to detect a correlation between BP drop or elevation, acceleratoin or slowing of LBF and onset of cardiac rhythm disturbances.

The results of these studies revealed that, according to ECG analysis, Macaca rhesus monkeys develop various functional disturbances referable to cardiac conduction and automatism during exposure to +Gz using protocols 1 and 2.

As we know, the following factors play a part in the genesis of changes in cardiac rhythm with exposure to accelerations: displacement of the heart in the chest under the influence of inertial forces, change in delivery of blood to the chambers of the heart, myocardial hypoxia, reflex influences arising as a result of system and regional circulatory disturbances, change in tonus of autonomic regulatory centers of the cardiovascular system [1, 3].

Apparently, change in tonus of extracardiac nerves, which occurred as a result of emotional stress (rotation without protective cabin) and reflex influences from different organs and systems of the body play the principal role among the above-mentioned factors in our studies. This is indicated by the fact that analogous disturbances of cardiac rhythm appeared at low levels (up to 2 G) during the preliminary rotation sessions and with exposure to 5–10 G in the main sessions. These disturbances appeared both in the presence of increased tonus of the sympathetic nervous system (marked sinus tachycardia) and with increase in parasymepathetic nervous system tonus (development of relative bradycardia).

Our findings revealed that wide individual differences in tolerance to +Gz accelerations, as well as differences in responses of circulatory parameters to this factor, are inherent in Macaca rhesus monkeys. This applied in particular to the BP response. In some animals, BP remained elevated throughout the period of the study, whereas others developed a brief hypotensive reaction. Unlike Yu. Ye. Moskalenko et al. [2], we did not observe decline of BP in the carotid artery of 20–30 mm Hg per G

of +Gz accelerations. Evidently, this is attributable to methodological differences in conducting the experiments: the above function was obtained in an acute experiment with animals whose great vessels were in horizontal position.

The LBF changes in the carotid of monkeys exposed to +Gz were all in the same direction; however, their severity varied on a strictly individual basis, ranging from an insignificant decline (10–15%) to total, although brief, arrest of blood flow.

On the whole, the response of the monkeys' cardiovascular system to +Gz accelerations indicates that there are well-developed compensatory mechanisms in waking Macaca rhesus monkeys, and they provide for rapid normalization of hemodynamic parameters in the aftereffect period.

Considering the results of these studies, animals with good tolerance to +Gz accelerations were recommended for use in an experiment aboard Cosmos-1514, and this was instrumental in the successful completion of the experiment.

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EFFECT OF LOW-FREQUENCY WHOLE-BODY VERTICAL VIBRATION ON THE SEROTONINERGIC SYSTEM OF THE BRAIN AND SPINAL CORD

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 22, No 1, Jan–Feb 88 (manuscript received 8 Apr 86) pp 45–49

[Article by A. S. Dmitriyev and G. K. Tropnikova]

[English abstract from source] Rat experiments were performed to study variations in serotonin (5-HT) and its metabolite (5-hydroxy indole acetic acid) in different CNS compartments. Control animals were exposed to an acute vibration stress (10 Hz, 1 mm, 2 m/sec^2, 15 min) and experimental animals to a prolonged (52–54 days) vibration test. Acute vibration led to 5-HT activation which was most significant in the hippocampus, diencephalon, cerebellum and in the sacrolumbar cord. Prolonged viration caused an increase of 5-HT in the parietal cortex and its enhanced utilization in the striatum, diencephalon, pons and in the sacrolumbar cord. As compared to the controls, vibration produced a smaller accumulation of 5-HT in the hippocampus and a larger accumulation in the cerebellum, diencephalon, medulla oblongata and spinal cord. The paper discusses the role of regional changes in 5-HT metabolism and reactivity of serotoninergic structures in the mechanism of vibration-related somatosensory disorders.

[Text] The low-frequency, generalized, vertical vibration (LFV) generated by modern transport (aviation and navy, large-tonnage trucks, railroad, etc.) makes it difficult to monitor instrument readings, lowers precision and coordination of movements, thereby intensifying nervous and emotional stress, which leads to rapid fatigue and decline of work capacity of crews [2, 3, 5, 6, 21]. Here, a special role is attributed to functional impairment of analyzers—vestibular, visual and motor [10]. However, the central neurohumoral mechanisms on which it is based, the role of monoaminergic systems (including the serotoninergic one) and the effect of vibration as a function of adaptability have been insufficiently investigated. The information in the literature concerning changes in concentration of serotonin (5-hydroxytryptamine—5-HT) in blood and excretion of its metabolite, 5-hydroxyindoleacetic acid (5-HIAA), in urine of patients with vibration sickness is indicative of existence of a relationship between neurovascular disorders and extent of impairment of 5-HT metabolism [14].

Aside from involvement of regulation of both cerebral [18] and systemic [9] circulation, 5-HT has an activating effect on the hypothalamus-hypophysis-adrenal system [9], it

plays an important part in enhancing nonspecific constitutional resistance. A selective effect of 5-HT on various afferent signals—proprioceptive, tactile, interoceptive [15, 19, 22, 27]—was discovered on the level of the spinal cord; the flow of such signals increases dramatically under the effect of LFV as a result of adequate excitation of mechanoreceptive systems. At the same time, there is no information in the literature concerning postvibration changes in 5-HT metabolism in the spinal cord. Yet it is expressly here that the first stage of integration of ascending heteromodal sensory information is effected toward higher centers of regulation of somatic and autonomic functions—cerebellum, thalamus, hypothalamus, caudate nucleus, hippocampus, cerebral cortex, which are monosynaptically innervated by serotoninergic neurons of nuclei of the mesencephalic sutura, pons and medulla oblongata[1].

We submit here the results of analysis of changes in 5-HT and 5-HIAA content of the above-mentioned parts of the brain in response to acute vibration stress, in control animals and those exposed to chronic LFV. This approach enabled us to avoid leveling off of data, which is possible when examining the entire brain [13, 16]. As compared to the method of assaying 5-HT alone [10, 17], it furnishes more information about processes in regulatory centers responsible for sensorimotor coordination and formation of compensatory and adaptive reactions.

Methods

Experiments were conducted on white female Wistar rats with average weight of 250–280 g. The 1st group consisted of vivarium control animals (control group), the 2d—rats exposed to whole-body vertical vibration on a VDS-200A vibration table for 52–54 calendar days, 4 h/day, 5 times a week (experimental group); vibration parameter were 10 Hz frequency, 1 mm amplitude and 2 m/s^2 rate of acceleration. Noise from the vibration table was at a level of 62 dB.

Each group was divided into 2 subgroups of 8–10 animals to assess distinctive features in reactivity of the serotoninergic system. The rats of one subgroup were put in individual box-cages and exposed to test vibration (TV) for 15 min on the same vibration table. The second subgroup of rats confined in analogous cages were put next to the vibration table and used to obtain baseline data.

After decapitating the animals, the brain was extracted rapidly, washed in glacial 0.9% NaCl solution and subsequent preparation was performed under refrigeration.

5-HT and 5-HIAA levels were measured in the thoracic and lumbosacral regions of the spinal cord, medulla oblongata, pons, midbrain, cerebellum, hypothalamus, thalamus, caudate nucleus, hippocampus and parietal cortex using a previously described method [20]. The obtained data were submitted to statistical processing. Reliability was assessed using Student's t criterion.

Results and Discussion

In the 1st group of animals, TV elicited elevation of 5-HIAA level in most of the tested regions of the brain, which was associated (with the exception of the diencephalon) with increase in 5-HT content (Figure 1a). This is indicative of activation of central serotoninergic structures, the extent of which varied significantly in different parts of the CNS [central nervous system]. The least change in indole was noted in the midbrain and medulla, where the bodies of serotoninergic neurons are concentrated,

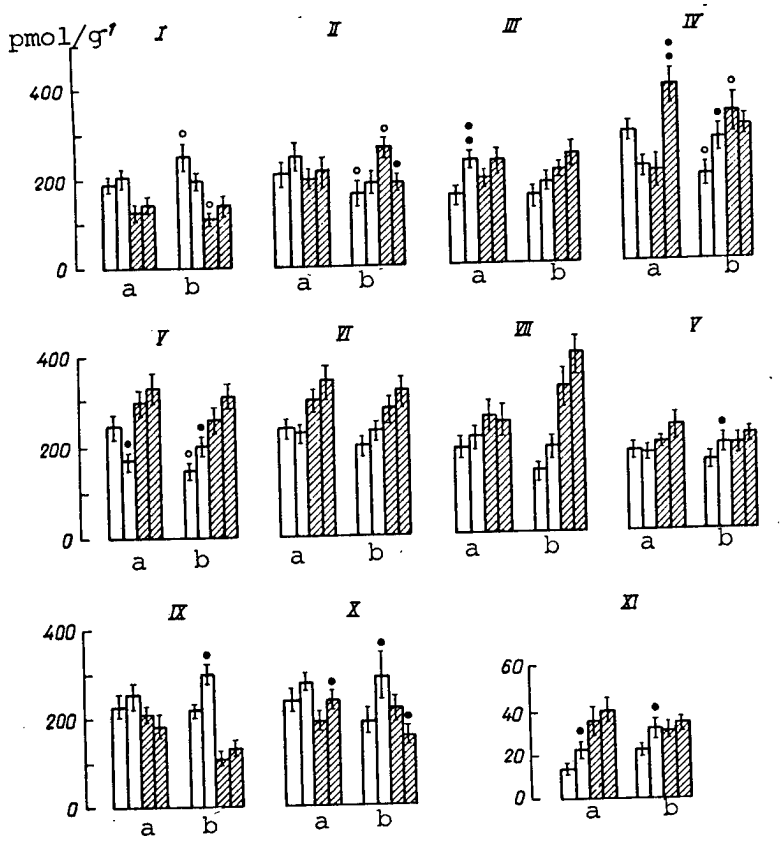

Figure 1. Effect of TV on 5-HT and 5-HIAA content in some parts of the brain and spinal cord of control (a) and experimental (b) groups of animals

White bars—5-HT; hatched—5-HIAA. For each substance, the left bar refers to the baseline and the right, to TV

I) parietal region of cerebral cortex
II) striatum
III) hippocampus
IV) hypothalamus
V) thalamus
VI) mesencephalon
VII) pons
VIII) medulla
IX) thoracic region of spinal cord
X) lumbosacral region of spinal cord
XI) cerebellum

•$p<0.05$, ••$p<0.01$ between TV and baseline for each substance; o—$p<0.05$ between baselines **a** and **b**

whereas in terminal regions they were found in the parietal cortex and caudate nucleus, which is consistent with the data in [17]. But, unlike the males studied in [17], the females showed the most significant changes in the hippocampus and hypothalamus. The elevation of 5-HT level (+49.5%) was greater than that of 5-HIAA in the hippocampus, whereas in the hypothalamus dramatic rise of 5-HIAA (+89.3%) was associated with decline in 5-HT, probably due to increased utilization of the latter. The fact that activation of serotoninergic neurons of the hippocampus and hypothalamus stimulates production of ACTH and corticosteroids [11] compels us to believe that the changes in 5-HT and 5-HIAA observed in the hippocampus and hypothalamus constitute a typical response to stress and they are adaptive, aimed at mobilization of defense mechanisms. The elevation of corticosterone level observed in plasma of Wistar rats with analogous LFV parameters [16] can serve as confirmation of this.

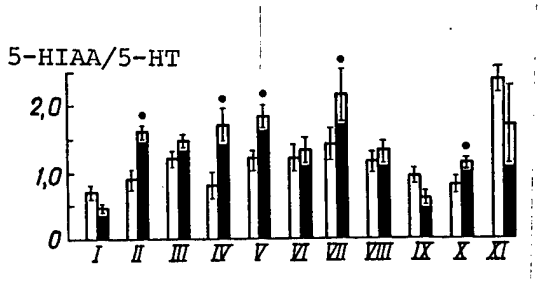

Figure 2.
Effect of long-term vibration on ratio, 5-HIAA/5-HT in some parts of brain and spinal cord of adult female rats
White bars—control; black—vibration. •$p<0.05$ in relation to control; other designations are the same as in Figure 1

In the spinal cord, the most TV-reactive structures were the serotoninergi ones in the lumbosacral region, where we observed concurrent increase in 5-HT and 5-HIAA. Considering the data indicative of the inhibitory effect of 5-HT on conduction of interoceptive signals, in particular, cardiopulmonary [15], tactile [22] and nociceptive [19, 27], and its enhancing effect on conduction of proprioceptive [22] afferent signals, it can be concluded that activation of serotoninergic structures of the lumbosacral cord, which was observed in response to LFV, is instrumental in conducting the most important sensory information to the body's most important centers for coordination of complex motor acts—the hypothalamus and cerebellum. In the hypothalamus, which is one of the main relay stations on the way to sensory signals for the caudate nucleus and cerebral cortex, there was prevalence of processes of utilization of 5-HT, as indicated by rise of 5–HIAA/5-HT index, an indirect indicator of degree of metabolism and utilization of 5-HT. Conversely, in the cerebellum there was intensive accumulation of 5-HT (+44.4%). Since 5-HT has predeominantly an inhibitory effect on cerebral neurons [24], its reliable elevation in the cerebellum could be one of the causes of the impaired oculomotor reactions observed during vibration [3, 6] as a result of possible depression of activity of Purkinje cells, which are a mandatory element of feedback in the system for control of the vertical vestibulo-ocular reflex [12].

In animals submitted to long-term vibration, there were changes in the baseline state of the serotoninergic system. First of all, we observed a change in 5-HT metabolism in the anterior parts of the brain—parietal cortex, caudate nucleus and hypothalamus (Figure 1b). We also observed reliable increase in 5-HT content, whereas its level was low in the caudate nucleus and hypothalamus, probably due to intensification of its utilization, since the concentration of 5-HIAA was higher than in the corresponding baseline for control animals. In spite of the fact that the levels of the tested substances did not differ from the control in other parts of the brain, the 5-HIAA/5-HT index for the thalamus, pons and lumbosacral region of the spinal cord was higher in rats submitted to long-term vibration, and it was indicative of some activation of 5-HT metabolism in these parts of the brain (Figure 2)

The opinion is held that, on the cortical level, 5-HT is involved in screening afferent signals arriving via specific pathways and enhances systemic reactivity to sensory stimuli [4]. Since the parietal region of the cerebral cortex is the cortical representation of the vestibular analyzer in rodents [25], the high 5-HT level in this part of the cortex in animals submitted to long-term vibration and the overt tendency toward its decline under the influence of TV could serve as one of the possible explanations for the change in conduction of the vestibular analyzer observed under the effect of long-term vibration [10].

Unlike the parietal region of the cerebral cortex, TV elicited elevation of 5-HT in other tested regions of the brain of the experimental group of rats, including the hypothalamus and thalamus. However, it should be noted that there was almost 2-fold

decrease in elevation of 5-HT level in the hippocampus. Conversely, it showed a more significant elevation, as compared to the control group, in the cerebellum (+66.8%), thoracic (+37.6%) and lumbosacral (+50.7%) regions of the spinal cord, as well as in the medulla oblongata (+27.2%). This was associated with decline of 5-HT catabolic processes in the striatum and lumbosacral region of the spinal cord. This was indicated by the reliable decline of 5-HIAA content in the latter, by 28.4 and 26.8%, respectively.

Thus, our findings indicate that not only the functional state of the brain's serotoninergic system, but its reactivity to TV change in rats submitted to long-term vibration. The predominant reaction is elevation of 5-HT content, which is the most significant in the cerebellum and spinal cord. This explains, in part, the decrease in some forms of sensibility caused by vibration, for example, nociceptive [7, 10]. Of course, one must bear in mind the data concerning the inhibitory effect of 5-HT on the nociceptive neurons of the dorsal cornua of the spinal cord [19]. In addition, excessive accumulation of 5-HT in the CNS and attenuation of its inactivation, particularly in the caudal regions of the brain and spinal cord, are probably among the causes oaf drowsiness and malcoordination of muscular activity observed during the long-term effect of vibration [6, 10]. Information concerning involvement of the large nucleus of the raphe of the medulla oblongata in formation of slow-wave sleep [23] and change, under the effect of 5-HT, in excitability of spinal motor neurons [26] could serve as the basis for such an assumption.

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UDC: 612.273.2+612.121.3]-612.115.014.46:615.357.452

DISTINCTIVE FEATURES IN BLOOD CLOTTING AND FIBRINOLYTIC PROPERTIES UNDER EFFECT OF EPINEPHRINE IN PRESENCE OF HYPOXIA AND HYPERCAPNIA

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 22, No 1, Jan–Feb 88 (manuscript received 6 Nov 86), pp 49–53

[Article by G. D. Pak, V. S. Sverchkov, T. N. Danilevskaya and T. P. Trandafilova]

[English abstract from source] Acute experiments were carried out on 50 dogs to study the effect of epinpehrine in hypoxic (N_2—15 to 10% 0_2) or hypoxic-hypercapnic (N_2—10%, 0_2—5% CO_2) atmospheres. Epinephrine led to a maximum increase of blood coagulation and fibrinolysis in normoxic atmosphere. Hypoxia reduced the shift of most hemostasis parameters in response to epinephrine. However, in N_2—10% 0_2 atmosphere the epinephrine-induced increase of blood coagulation was superimposed on initial hypoxic hypercoagulation and caused serious disorders in hemostasis. In hyperoxic-hypercapnic atmosphere increase of blood coagulation in response to epinephrine was more than doubled when compared to that in hypoxic atmosphere, reaching control values. Nevertheless, after epinephrine administration the ratio of coagulatory, anticoagulatory and fibrinolytic activities was more beneficial in hypoxia-hypercapnia and the coagulation potential was lower than in hypoxic or normoxic atmospheres.

[Text] Data to the effect that hypoxia and hypercapnia are capable of altering the responses of various functional systems to additional stimuli (hypokinesia, pharmacological agents, "ascent" in an altitude chamber, etc.) are important to clinical physiology and medicine [1, 12, 14]. At the same time, we found no information about the distinctions of homeostasis system responses to experimental hypoxia and hypercapnia. Our objective here was to determine the dynamics of blood coagulation and fibrinolytic properties under the effect of epinephrine in the presence of varying degrees of hypoxia and the combined effect of hypoxia and hypercapnia. We chose epinephrine as the indicator of reactivity and potential of the blood clotting and fibrinolytic systems because of its capacity to stimulate hypercoagulation and hyperfibrinolysis [4, 6, 7].

Methods

Acute experiments were performed on 50 mongrel dogs weighing 5–15 kg under hexenal anesthesia breathing hypoxic mixtures with 15, 14, 13, 12% 0_2 in nitrogen (p_a0_2

64–62 mm Hg), with 10% O_2 in nitrogen (p_aO_2 42–21 mm Hg) and hypoxic-hypercapnic mixture containing 10% O_2 and 5% CO_2 (P_aO_2 68–42 mm Hg). Epinephrine was given intravenously in a dosage of 0.1 mg/kg weight in 1 min after 25-min breathing of atmospheric air (control) and each gas mixture. Blood was drawn from the lateral branches of the femoral artery before giving epinephrine and 1 min after it. Coagulation hemostasis was investigated using methods described in manuals [3, 9]. Oxygen tension was measured on a biological microanalyzer. The data were submitted to statistical processing with use of Student's criterion and coefficient of correlation (r).

Results and Discussion

The results of our experiments revealed that, before injection of epinephrine, the hemostasis system was characterized by a tendency toward increased coagulability of blood under the effect of 24-min hypoxia with decline of p_aO_2 to 42 mm Hg, and reliable hypercoagulation with decline of p_aO_2 to less than 42 mm Hg. The most marked hypercoagulation change under the effect of epinephrine was observed when the animals breathed atmospheric air, and shortening of silicone test coagulation time constituted 113 s. Hypoxia reduced to more than one-half acceleration of plasma silicon time in response to epinephrine: it constituted 43.7 s when breathing the mixtures with 15–12% O_2 and 52.6 s when using the mixture with 10% O_2 (see Table).

The significant reduction after giving epinephrine in koalin test coagulation test time for plasma rich and poor in platelets (by 25.2 and 40.4 s, respectively in the control, 13.0 and 21.5 s with use of mixtures with 15–12%O_2, 11.3 and 18.1 s with use of mixture with 10%O_2) was associated wtih decrease in difference between them, from 34.7 to 19.5 s in the control, from 30.2 to 21.7 s when using mixtures with 15–12% O_2, from 29.6 to 22.9 s when using the mixture with 10% O_2. These findings, along with the reliable 9.2% decrease in index of release of thrombocyte activators (IRTA) in the control and a tendency toward its decline in an altered gas environment under the effect of epinephrine, confirm the conception that thrombocytes, which release fragments of cytoplasmic membranes with thromboplastic properties into the blood stream when interacting with catecholamines, play a substantial role in development of the hypercoagulemic response to epinephrine [4, 6, 8]. On the other hand, our data indicate that hypoxia limits the extent of influence of platelets on blood clotting in response to increase in blood epinephrine concentration.

The decrease in index of range of contact activation (IRCA), by 7.2% in the control and by 0.4 and 1.5% at p_aO_2 64–42 and 42–21 mm Hg, respectively, reflects intravascular activation of the contact phase of coagulation which, in the opinion of a number of authors [4], is effected by means of direct interaction between epinephrine and contact phase factors. However, the intensity of this process diminishes significantly under hypoxic conditions, as indicated by our findings.

In addition to intensification of procoagulant activity, we observed marked increase in activity of antithrombin III after administration of epinephrine in the control (by 19.1%) and less marked under hypoxic conditions (by 9.3 and 5.2% at p_aO_2 64–42 and 42–21 mm Hg, respectively). Under these conditons, heparin concentration had a tendency toward decrease, reaching lowest values at p_aO_2 42–21 mm Hg.

Thrombin time and fibrinogen concentration showed virtually no changes in all cases after injection of epinephrine. Activity of the fibrin-stabilizing factor increases, the maximum change (by 19.7 s) in response to epinephrine being observed when we used

Parameters of blood clotting and fibrinolytic activity in response to epinephrine under hypoxic and hypoxic-hypercapnic conditions (M±m)

Parameter	Before epinephrine				After epinephrine			
	control	hypoxia		hypoxia+ hypercapnia	control	hypoxia		hypoxia+ hypercapnia
		P_aO_2 64–42 mm Hg	P_aO_2 42–21 mm Hg	P_aO_2 68–42 mm Hg		P_aO_2 64–42 mm Hg	P_aO_2 42–21 mm Hg	P_aO_2 68–42 mm Hg
Plasma silicone time, s	233,1±4,6	220,8±11,1	179,2±7,9	246,9±11,1	120,1±16,3*	177,1±12,1*	126,6±7,7*	147,7±7,7*
kaolin	63,8±1,1	63,4±0,3	53,1±2,4	64,3±2,5	38,6±2,3*	50,4±2,5*	41,8±2,4*	44,3±2,2*
Thrombocyte-poor plasma KT, s	98,5±2,2	93,6±3,4	82,8±3,7	94,4±4,0	58,1±3,4*	72,1±4,0*	64,7±4,8*	67,1±4,3*
IRCA, %	71,5±0,7	70,1±1,1	68,8±1,2	73,1±1,1	64,3±3,7	69,7±1,5	67,3±2,0	68,2±1,1
IRTA, %	34,9±1,3	31,9±2,4	33,8±1,7	31,0±2,0	25,7±3,7	29,2±1,8	34,0±3,5	30,4±2,1
Thrombin time, s	17,4±0,4	18,2±1,1	16,5±0,7	16,8±0,8	16,5±1,0	18,2±1,1	17,1±1,6	16,7±1,0
Fibrinogen, mg%	342,7±13,4	345,3±38,0	392,1±18,2	372,2±23,5	323,5±43,8	350,3±36,5	387,7±31,3	342,1±34,4
Factor XIII, s	81,4±7,4	77,6±9,1	51,2±3,1	74,8±5,4	92,9±7,1	87,8±12,9	70,9±8,2	87,4±13,2
Free heparin, s	7,9±0,4	8,4±0,7	6,6±0,4	7,9±0,8	—	7,0±0,6	5,9±1,1	7,2±0,9
Antithrombin, activity, %	117,2±3,0	111,3±4,5	116,5±6,9	131,1±4,5	136,3±14,1	120,6±4,7	121,7±8,7	122,4±4,6
Nonenzym. fibrinolysis, %	16,2±1,6	20,0±2,2	21,5±4,5	23,0±3,2	11,2±5,4	22,5±4,7	7,7±4,5*	18,2±4,4
Fibrinolysis, %	28,5±2,0	30,1±4,4	26,2±4,9	37,6±6,7	95,3±4,7*	76,8±5,8*	59,1±7,3*	64,9±8,9*

*Reliable differences as compared to baseline. KT) kaolin time

the mixture with 10% 0_2. However, due to the preliminary decrease in activity of factor XIII under the effect of such marked hypoxia, its values were lower than in control experiments.

After administration of epinephrine, we demonstrated a 5% decrease in nonenzymatic fibrinolysis in the control. Under hypoxic conditions, with p_a0_2 under 42 mm Hg, it was dramatically depressed (by 13.8%), which could be attributable to the low concentration of free heparin in blood. With p_a0_2 at 64–42 mm Hg, there was virtually no change in nonenzymatic fibrinolysis. The combination of depression or depletion of anticoagulation system reserve with significant intensification of coagulating activity in the presence of acute hypoxia (10% 0_2 in nitrogen) and in the control, conditions are formed for thrombinogenesis and intravascular blood clotting. Indeed, in such cases a positive ethanol test was observed the most often.

Enzymatic fibrinolytic activity in response to epinephrine was characterized by maximum (66.8%) increasein the control and less marked under hypoxic conditions, and as hypoxemia increased the increment in fibrinolytic activity decreased to 46.7% at P_a0_2 of 64–42 mm Hg and to 32.9% at 42–21 mm Hg. Release of plasminogen activators from the vascular wall and formed blood elements is of particular significance to the increase in fibrinolytic activity of blood with injection of epinephrine [7, 13]. Perhaps, hypoxia, which alters the functional state of the latter, disrupts the process of release of fibrinolysis activators into the blood stream in response to epinephrine stimulation.

With addition of 5% CO_2 to the hypoxic mixture with 10% 0_2. we failed to observe activation of blood-clotting properties which is inherent in such a level of "pure" hypoxia; there was some increase in activity of antithrombin III and fibrinolysis, which provided on the whole for an optimum liquid state of blood. Under such conditions, plasma silicone time decreased

by 99.2 s, kaolin time for thrombocyte-rich and -poor plasma decreased by 20.0 and 27.3 s, respectively, in response to epinephrine; the difference between the latter under the effect of epinephrine decreased from 30.1 to 22.8 s, IRCA decreased by 4.8% and IRTA by 0.6%, i.e., reactivity of the above parameters under hypoxic-hypercapnic conditions was substantially greater than under purely hypoxic conditions, but lower than in the control.

Thrombin time and fibrinogen concentration did not change, while factor XIII activity increased by 12.7 s.

Absence of decrease in free heparin and some decline in activity of antithrombin III (by 8.7%), the level of which was the same as after injection of epinephrine in the presence of "pure" hypoxia, were typical of the action of epinephrine under hypxoic-hypercapnic conditions.

After administration of epinephrine in the case of combined exposure to hypoxia and hypercapnia, the parameters of both nonenzymatic and enzymatic fibrinolysis were higher than with use of a breathing mixture with 10% 0_2, although we did observe the lowest increase in enzymatic fibrinolysis (by 27.3%) under the effect of epinephrine in the presence of hypoxic-hypercapnic conditions. Consoequently, there was an optimum correlation between coagulation, anticoagulation and fibrinolytic activity of blood after injection of epinephrine under hypoxic-hypercapnic conditions, while the coagulation potential was lower than in the presence of analogous hypoxia without hypercapnia.

Analysis of our results revealed that a maximum increase in coagulating and fibrinolytic activity of blood with use of epinephrine is observed when breathing atmospheric air. Hypoxia diminishes changes in the vast majority of hemostatic parameters in response to epinephrine, as a result of which coagulation and fibrinolytic potentials of blood do not reach the control level. We demonstrated a rather close correlation between plasma silicone time after giving epinephrine and degree of hypoxemia ($r=0.75$) and plasma silicone time before administration of epinephrine ($r=0.66$), i.e., the greater the baseline coagulability of blood and the lower the oxygen tension in arterial blood, the higher the coagulation potential of blood following administration of epinephrine. The results of these experiments are consistent with data in the literature [5, 7] to the effect that the nature of changes in blood-clotting and fibrinolysis systems under the effect of additional stimuli depends on their baseline functional state and condition of regulatory systems. Evidently, both hypercoagulation , which occurs with a breathing mixture containing 10% 0_2 (p_a0_2 42–21 mm Hg), and stimulation of the adrenosympathetic system, which is observed with this degree of hypoxia [10], are capable of limiting additional increase in clotting properties of blood under the effect of exogenous epinephrine. Nor can we rule out the possibility that a dramatic decline of p_a0_2 to 42–21 mm Hg, which leads to accumulation in blood of incompletely oxidized metabolic products [11], could diminish sensitivity of adrenoreceptors [2] and the capacity of the vascular wall and formed blood elements to release thromboplastic substances and fibrinolysis activators into the blood stream under the influence of epinephrine. At the same time, the absence of increase in blood epinephrine concentration [10, 14], reliable change in coagulability of blood or disturbances referable to aerobic metabolsim when inhaling mixtures with 15–12% 0_2 (p_a0_2 64–42 mm Hg) warrant the belief that there are some other mechanisms that limit activation of blood-clotting under the effect of epinephrine under such conditions.

When using a breathing mixture with 10% O_2 and 5% CO_2, acceleration of blood-clotting under the effect of epinephrine exceeded by more than two times the level observed with "pure" hypoxia and was close to control levels. Nevertheless, the baseline functional state of the hemostasis system under hypoxic-hypercapnic conditions was instrumental in maintaining a lower coagulation potential of blood than in the control and with analogous "pure" hypoxia, and it enhanced resistance of the hemostasis system to the deleterious effect of epinephrine.

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HEMORRHAGES AND HEMOSTASIS IN GUINEA PIGS EXPOSED TO RADIATION AT HIGH ALTITUDE

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 22, No 1, Jan–Feb 88 (manuscript received 22 Jan 87) pp 53–57

[Article by V. N. Tartakovskiy and S. B. Daniyarov]

[English abstract from source] Hemorrhagic intensity, hemostasis and blood vessel wall resistance to mechanical effects were studied in guinea-pigs exposed to whole-body irradiation (3.0 Gy). The animals were irradiated at low altitude (760 m above sea level) and at high altitude (3200 m above sea level) after 1 and 31 days of adaptation. It was demonstrated that hemorrhagic intensity in both groups of guinea-pigs irradiated at high altitude was significantly reduced in comparison with that at low altitude. The decrease in radiation induced hemorrhages at high altitude is associated with less severe changes in thrombopoiesis, blood vessel wall and blood coagulation.

[Text] Hemorrhages and bleeding occur at the height of acute radiation sickness in man and animals, and they largely determine the course and outcome of this sickness [3, 5, 8]. Heretofore, the distinctions of hemorrahges in the presence of radiation sickness, which occurs and proceeds at high altitude, had not been investigated. For this reason, we studied the severity of radiation-related hemorrhages and changes in the hemostasis system of guinea pigs adapting to alpine climate.

Methods

We used 250 guinea pigs weighing 300–400 g in this study. We conducted 5 series of experiments: I—control (in Frunze), II—radiation at low altitude (Frunze, 760 m above sea leve), III—adaptation to alpine climate (Tyan-Shan, Tuya-Ashu pass, 3200 m above sea level), IV—radiation at high altitude after 24-h adaptation, V—radiation at high altitude after 31st day of adaptation. Irradiated animals were examined on the 1st, 3d, 10th and 30th days of radiation sickness. We had a control at high altitude for each group of irradiated animals, i.e., adapting guinea pigs were examined on the 2d, 4th, 11th, 31st, 41st and 61st days of adaptation. Hemorrhagic signs and condition of hemostasis system were studied in an acute experiment, for which anesthetized animals (nembutal, 50 mg/kg) were sacrificed at different intervals following exposure to radiation in a dosage of 3.0 Gy (^{137}Cs, dose rate 0.139 mGy/s). Hemorrhages were examined macroscopically after dissecting the animals on the 10th postradiation day.

We measured the perpendicular dimensions of hemorrhages and, using the formula for the area of an ellipse, we calculated the area occupied by effusions of blood in subcutaneous fatty tissue, muscles, cecum, stomach, gallbladder and urinary bladder. We determined the volume of the hemorrhages in the lungs. A point scoring system was used for the intestine (with the exception of the cecum), pancreas, testes and mesentery lymph nodes. For example, absence of hemorrhages in the intestine was given a rating of 0, sparse petechiae—1, petechiae over the entire course of the intestine—2, petechiae over the entire intestine in the presence of large (up to 2–3 mm in diameter) hemorrhages—3. We assessed thromocyte hemostasis according to extent of ADP-induced aggregation of platelets [17], index of spontaneous intravascular aggregation of thrombocytes (ISIA) [24] and platelet concentration in blood [18]. Coagulation hemostasis was evaluated acording to thromboelastography (TEG) readings—R, K and MA, thrombin time [16], fibrinogen concentration [13], antithrombin III (AT III) activity [15], ethanol [20] and protamine sulfate [22] tests. Resistance of the vascular wall was determined by the cup test.

Results and Discussion

In intact guinea pigs taken to the mountains, we observed effusion of blood in the lungs and wall of the cecum reaching several millimeters in size at all stages of 2-month adaptation. The most marked hemorrhages were seen on the 4th day of adaptation. Such changes were associated with decrease in vascular wall resistance, hypercoagulation changes in the hemostasis system, decrease in AT III sactivity and presence of products of fibrin degradation (PFD) in the blood (Table 1), i.e., in guinea pigs, the process of adaptation to alpine climate is associated with the thrombohemorrhagic syndrome (THS).

Adrenalinemia [7, 9] and related activation of the contact phase of blood-clotting, thrombocytes and depression of anti-aggregation activity of the vascular wall [2, 3, 11] may be the cause of THS in the mountains. Intensification of lipid peroxidation in the mountains, which elicits damage to the endothelium [19], decline of AT III activity [21] and imbalance in the prostacycline-thromboxane system [23], is another predisposing factor for THS. Morphologically, epithelial desquamation is observed at high altitudes [4, 6] which leads, on the one hand, to release of thromboplastin into the blood stream and, on the other hand, to activation of the contact phase of coagulation of blood. In addition, it has been shown that tissue thromboplastin is discharged from the vascular wall in the presence of hypoxia [10]. Authors have related the possibility of release of tissue thromboplastin into the blood stream to increase in vascular permeability under hypoxic conditions [10, 12].

In guinea pigs, altitude THS is characterized by a lengthy hypercoagulemic phase. Prolonged hypercoagulemia and related presence of microclots of fibrin and aggregates of platelets in the blood stream (high ISIA) alter microcirculatory conditions, make it difficult for blood to pass in small vessels and, consequently, cause tissue, in particular the vascular wall, to receive a short supply of nutrients and oxygen, in the face of already existing hypoxia at high altitude. This leads to dystrophic processes in vascular wall tissues and decrease in its resistance to mechanical factors. Such avascular wall can be easily injured when organs move (peristalsis in the cecum, ventilation in the lungs). They are instrumental in rupture of vessels and hemorrhages in the lungs, centralization of blood and pressure elevation in the pulmonary circulation, which are observed at high altitudes.

Table 1. Hemostasis and resistance of vascular wall of animals at different stages of adaptation to high altitude

Parameter	Lowlands	Day of altitude adaptation					
		2	4	11	31	41	61
Cup test (number of petech.)	5,5±0,9	7,3±0,9	10,5±1,2*	11,0±0,8*	21,0±4,0*	15,2±2,6*	20,2±2,6*
Platelet concentr. ($\cdot 10^9$/ℓ)	568±21	558±31	690±45*	493±31	426±28*	512±28	535±33
ISIA, relative units	1,09±0,02	1,33±0,05*	1,31±0,04*	1,28±0,04*	1,28±0,04*	1,26±0,04*	1,29±0,04*
ADP-induced platelet aggregation, % TEG:	75,8±0,7	79,6±0,6*	81,3±0,9*	80,2±1,0*	78,2±1,2	82,3±0,9*	81,1±0,7*
R, s	346±15	350±17	287±19*	345±24	282±42	236±16*	307±13*
K, s	152±8	123±9*	125±8*	124±10	118±9*	106±8*	133±10
MA, mm	60,5±1,5	71,1±2,7*	74,1±2,3*	72,7±0,9*	69,7±2,4*	71,0±1,9*	70,8±1,1*
Thrombin time, s	15,2±0,2	11,7±0,2*	12,5±0,1*	13,7±0,1*	13,6±0,1*	13,8±0,1*	13,5±0,2*
Fibrinogen concentr., g/ℓ	3,54±0,08	5,77±0,33*	5,66±0,3*	4,62±0,25*	4,0±0,17	4,14±0,06*	3,86±0,13
Antithrombin III activity, s	32,1±0,4	22,6±1,2*	23,8±0,9*	25,0±0,5*	24,6±0,6	25,1±0,5*	24,8±0,4*
Ethanol test, %	0	100	100	100	50	33	33
Protamine sulfate test, %	0	0	0	0	0	0	0

Here and in Tables 2 and 3: *$p<0.05$, as compared to "lowland" animals.

Table 2. Hemostasis and resistance of vascular walls in animals exposed to radiation at high and low altitudes

Parameter	Series of experiments								
	I			IV			V		
	postradiation days								
	1	3	10	1	3	10	1	3	10
Cup test (numb.petech.)	2,1±0,5	7,3±0,9	38,5±5,8	6,5±0,9*	9,7±0,5*	24,1±3,2*	15,5±1,8*	22,7±1,9*^{c}	37,1±2,7^c
Thrombocyte concentration ($\times 10^9$/ℓ)	568±21	467±20	17,0±2,3	454±18*	547±20*	34,3±4,5*	531±18^c	513±20	44,3±3,1*
ISIA, relative units	1.50±0,04	1,42±0,02	1,12±0,04	1,69±0,04*	1,48±0,03	1,11±0,05	1,43±0,03^c	1,41±0,03	1,09±0,04
ADP-induced platelet aggreg., % TEG	79,5±0,5	80,0±0,4	—	80,7±0,4	82,6±0,6*	—	82,3±0,2*^{c}	82,5±0,1*	—
R, s	321±15	313±26	444±15	288±21	289±12	351±13*	351±11^c	261±15	377±17*
K, s	142±9	128±8	—	108±6*	105±11	—	134±14	113±10	—
MA, mm	73,6±0,7	70,0±1,1	4,4±1,7	78.6±1,1*	77,3±1,6*	22,0±2,3*	79,1±1,8*	80,7±1,5*	14,4±1,5*^{c}
Fibrinogen, g/ℓ	4,67±0,12	4,46±0,12	2,83±0,08	5,83±0,12*	4,71±0,25	4,25±0,08*	6,12±0,04*	4,96±0,25	4,29±0,04*
Antithrombin III, s	41,2±0,6	38,0±0,3	27,3±0,4	28,7±0,4*	27,6±0,7*	24,0±0,4*	32,8±0,4*^{c}	31,8±0,6*^{c}	23,8±0,4*
Ethanol test, %	100	58	33	100	100	100	100	100	83
Protamine sulfate test, %	16	42	66	16	16	33	0	0	33

^{c}--$p<0.05$, as compared to series IV.

In addition to the fact that onset of hemorrhages in the mountains is related to high-altitude THS, this syndrome is also interesting in another respect. The fact of the matter is that tissue hypoxia, which is associated with THS, is summated with hypoxia related to low oxygen pressure in alpine air. Thus, THS may be one of the mechanisms of enhanced radioresistance, which has been observed at high altitudes.

THS develops not only during adaptation to an alpine climate, but in response to radiation. Thus, on the 1st and 3d postradiation days in the lowlands, there is hypercoagulation according to TEG results, platelet aggregation and ISIA increase, the ethanol and protamine sulfate tests become positive. Among the distinctions of radiation THS, we were impressed by the higher than normal level of AT III activity. This is probably attributable to increased synthesis of AT III, since it is known that protein synthesis by the liver is intensified following irradiation [14].

When guinea pigs are exposed to radiation at high altitude (both after 1-month adaptation and without adaptation), parameters of TEG, thrombin time, AT III activity, platelet aggregation and ISIA are indicative of even more marked hypercoagulation changes at the early stages of radiation sickness than when they are irradiated in the lowlands (Table 2).

At the height of radiation sickness, all of the groups of irradiated animals showed dramatic decrease in vascular wall resistance, drop in platelet concentration in blood and depressed coagulation hemostasis. However, it should be noted that these parameters underwent less profound changes in guinea pigs irradiated at high altitude than with irradiation at low altitude.

The same applies to severity of radiation-related hemorrhages: in all groups of irradiated animals we observed hemorrhages at the height of radiation sickness; however, hemorrhages in virtually all organs were significantly fewer in specimens exposed to radiation at high altitude than in the lowlands. The only exceptions were the cecum and mesentery lymph nodes. There, there were somewhat (unreliable) more hemorrhages in the "high-altitude" animals than in those irradiated at low altitude. But the amount of bleeding in these organs was infinitesimal as compared to the massive effusions in subcutaneous fatty tissue, muscles and lungs. For this reason, the changes in the intestine cannot have an appreciable effect on the general severity of radiation-related hemorrhages (Table 3).

As we have already mentioned, in animals exposed to radiation at high altitude TGS is greater in intensity than in those irradiated at low altitude, since there is more abrupt activation of the hemostasis system at the early stages of radiation sickness in both groups of guinea pigs irradiated at high altitude than in those irradiated at low altitude. However, the opposite correlation is observed with regard to severity of radiation hemorrhages at the height of radiation sickness: there are fewer hemorrhages in animals irradiated at high altitude than in those exposed in the lowlands. Consequently, TGS does not play an appreciable role in onset of hemorrhages when animals are exposed to radiation in the mountains.

The latter thesis allows us to comprehend why an alpine climate not only fails to potentiate, it even attenuates radiation hemorrhages, although being in an alpine region *per se* is associated with development of hemorrhages. The fact of the matter is that, with adaptation to high altitude, the genesis of hemorrhages is related to TGS and concomitant disturbances referable to the microcirculation and vascular wall

resistance, whereas in the presence of radiation sickness occurring in the mountains TGS is of secondary significance in development of hemorrhages, and its more severe course in animals exposed to radiation in the mountains, as compared to those exposed in the lowlands is compensated by less marked radiation damage to thrombocytopoeisis, coagulation hemostasis and vascular wall.

Table 3. Severity of hemorrhages in different organs of guinea pigs on 10th day after exposure to radiation at high and low altitudes

Localization of hemorrhages	Series of experiments		
	II	IV	V
Subcutanoues fatty tissue, cm^2	19,91±1,74	12,00±2,15*	9,09±1,77*
Muscles, cm^2	8,83±1,48	3,34±0,66*	2,60±0,67*
Lungs, mm^3	164,4±28,0	12,05±1,94*	9,03±1,88*
Cecum, mm^2	65,5±16,8	72,27±19,48	77,61±17,55
Stomach, mm^2	70,8±15,0	9,02±2,04*	9,52±1,9*
Intestine, score	2,11±0,13	1,83±0,15	1,66±0.23
Pancreas, score	0,44±0,12	0,17±0,09	0,05±0,05*
Testes, score	1,2±0,2 (10)	0,5±0,17* (12)	0,33±0,17* (12)
Mesentery lymph nodes of intestine, score	0,32±0,03	0,44±0,05	0,43±0,05
Gallbladder, mm^2	3,13±0,58	0,52±0,18*	0,33±0,07*
Urinary bladder, mm^2	0,87±0,19	0,68±0,19	0,62±0,20

Note: The organs are listed in order of diminishing severity of hemorrhages; number of organs is given in parentheses.

Comparison of the two groups of animals exposed to radiation at high altitude revealed that a somewhat larger number of platelets is preserved in those adapted by the 10th day of radiation sickness, whereas coagulation hemostasis and the vascular wall of this group were, on the contrary, more affected than in nonadapted irradiated animals. There is no reliable difference in severity of hemorrhages between these two groups; however, mean extent of hemorrhages was less marked in the subcutaneous fatty tissue, muscles and lungs of adpated animals.

Thus, exposure to radiation at high altitude of both guinea pigs adapted for 1 month and unadapted animals is associated with less marked involvement of thrombocytopoiesis, hemostasis, and vascular wall, and substantial reduction of radiation hemorrhages, as compared to animals exposed to radiation at low altitude.

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EFFECT OF LONG-TERM INHALATION OF ACETIC ACID VAPOR ON SOME FUNCTIONAL PARAMETERS OF MAN

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[Article by V. P. Savina and B. V. Anisimov]

[English abstract from source] Test subjects were continuously exposed to acetic acid vapors which form a constant component of enclosed atmospheres. The inhalation time was 15 to 22 days at concentrations of 5, 10 and 15 mg/m^3 or 10 days at a concentration of 26 mg/m^3. Physiological parameters showed statistically significant changes at concentrations of 15 and 26 mg/m^3. It is suggested that the changes are not adaptive but have been produced by the adverse effect of acetic acid vapors on the human body. It is therefore concluded that the 15 mg/m^3 concentration is threshold and the 5 and 10 mg/m^3 concentrations are ineffective in terms of the tests used. The most sensitive method is measurement of hydrocarbons (C_2–C_5), especially ethylene, in the exhaled air.

[Text] Acetic acid (AA) is a constant element of the gas environment of manned closed environments. Man is the principal source of AA in a closed environment. AA is contained in sweat, exhaled air, excreta, and it is formed when storing soiled laundry. In addition, AA is discharged by some polymers. At least 90 mg/day volatile fatty acids are discharged into the atmosphere of a closed chamber per person in exhaled air and perspiration (C_1–C_6), about 80% of which is referable to AA [7, 10, 13].

Hygienic regulations of levels of toxic agents in the case of chronic exposure are based on data pertaining to threshold doses for different paramters and accumulation of the toxic effect. Extrapolation of results obtained in animal experiments to man requires verification using the clinical statistical method. However, this route of defining MPC [maximum permissible concentration] is virtually inapplicable for individuals working in closed environments (for example, cosmonauts). Use of a margin of safety for species-related differences in sensitivity to a toxic agent leads to major mistakes in validation of MPC. There are no experimental data in the literature concerning the effect of continuous inhalation of AA vapor on man. The results of studies pursued on animals have shown that volatile fatty acids have no allergenic, mutagenic or carcinogenic effects in moderate concentrations, while their cumulative effect and aftereffects are minimal [11, 12]. We are reporting here the results of a study of the effect of long-term inhalation of AA vapor on human functional parameters.

Methods

There are no indications whatsoever in the literature concerning the specificity of physiological changes elicited by vapors of acetic and other fatty acids in low, close to threshold concentrations. This made it necessary to use a large number of methods in order to increase the probability of detecting parameter that are the most sensitive to the effects of acetic acid vapor.[1]

Test conditions

Test No	Days in closed environm.	Exposure to AA vapor, days	AA vapor concentr., mg/m^3	Heat exposure, days
1	20	15 (3d to 18th day)	5±1	None
2	20	15 (3d to 18th)	10±1,5	None
3	40	22 (6th to 28th)	15±0,8	None
4	20	17 (3d to 20th)	15±0,5	16, 17 & 18th d
5	20	10 (10th to 20th)	26±1,5	6, 7, 8, 16, 17 & 18th d

Five tests were performed in a 24-m^3 closed chamber (see Table). We started with an AA vapor concentration of 5 mg/m^3, which is the MPC for work zone air (MPC_{wz}). In subsequent tests, the concentrations of AA vapor constituted 2, 3 and 5 times the MPC_{wz}. The MPC_{wz} concentration is ineffective with intermitent exposure (up to 8 h/day, but no more than 41 h/week). There are data to the effect that the toxic effect of substances can be manifested in the case of continuous exposure in a concentration equaling MPC [7, 13]. For this reason, we used successively increasing concentrations of AA vapor in order to avoid any appreciable worsening of the condition of healthy subjects.

The air conditioning and regenerating systems maintained a temperature in the range of 21±2°C, relative humidity at 50±2%, oxygen concentration of 21±2% and carbon dioxide at 0.5±0.1%.

Heat (33±2°C with relative humidity of 50±5%, duration shown in the table) was used for detection of latent physiological disturbances in tests 4 and 5.

In each test there were four people in the chamber. These were essentially healthy men 26 to 42 years of age; there was a total of 18 men, 2 of whom participated in tests 1 and 3.

The subjects were kept on a standard diet containing 3000 kcal. They worked out daily on a cycle ergometer for 20 min at 100 W power and performed a set of physical exercises.

We assessed the condition of their cardiovascular system and external respiration at rest and during pedaling at stepped increments. We recorded the resting ECG in 12 leads, pulse wave propagation time in the following segments: left heart—carotid

[1]Ye. N. Antropova, L. Kh. Bragin, V. I. Bychkov, T. I. Golubeva, V.V. Zhuravlev, A. A. Ivanov, S. M. Ivanova, S. K . Kalandarov, M. P. Kalandarova, I. V. Konstantinova, T. I. Kuznetsova, G. A. Manovtsev, L. N. Mukhamediyeva, Yu. A. Spasskiy and M. Ya. Yakovleva participated in the studies.

artery, radial and femoral arteries, and dorsal artery of the foot. The stepped test consisted of 3-min pedaling at (successively) 100, 150, 200, 250 and 300 W, while recording the condition of the cardiovascular system and external respiratory function. Gas exchange was measured at rest also. We calculated all of the principal parameters of respiratory function at rest and while pedaling.

Physical work capacity—PWC_{170}—was determined on the basis of 5-min exercise at 30 and 60% of the power corresponding to maximum oxygen uptake.

Thermal status was assessed from the results of taking body temperrature readings at 11 points and calculating the weighted mean temperature and heat content.

Comprehensive psychophysiological examination included self-appraisal of current mental status using the Supos-8 psychometric method, testing the speed of simple oculomotor response, determining productivity of logical arithmetic operations, concentration and stability of responses and dynamics of sense of time.

Speed of dark adaptation was determined with an ADM-U4.2 adaptometer using a 3-min method.

We assayed blood serum levels of total protein, protein fractions, total lipids, cholesterol, lipoprotein fractions, 11-HCS [hydroxycorticosteroids] and sugar; we measured urea, uric acid, creatinine, ammonia and 17-HCS in urine [2–4]. Red blood cell metabolism was tested: we determined glycolytic activity, ATP content, 2,3-diphosphoglycerate and reduced glutathione, activity of lactate dehydrogenase and glucose-6-phosphate dehydrogenase [14].

The following parameters were determined to monitor the immunity system: reactivity of thymus-dependent lymphocyutes, activity of T-T helpers and suppressors, activity of normal killers and B cells, and rheumatoid factor. We ran the allergological reaction for leukocyte inhibition and application test [5, 6].

Intensity of lipid peroxidation was determined according to levels of ethylene and other hydrocarbons (C_2–C_5) in exhaled air [15–17].

Reliability of changes in parameters was determined according to Student or Wilcoxson's nonparameteric criterion, depending on the nature of distribution of parameters.

Results and Discussion

The concentration of AA vapor was increased smoothly over a 24-h period in all of the tests. The subjects perceived an odor only in the first moment. They failed to report any unpleasnat changes in the nasopharynx or respiratory tract.

In tests 1 and 2, all tested physiological, psychophysiological and biochemical parameters remained on the baseline level.

Under optimum microclimate conditions and with insignificant pollution of the atmosphere [10], physical work capacity during regular exercise on the cycle ergometer did not diminish, on the contrary, it even improved somewhat [8, 9].

In this series of tests, the subjects' work capacity did not change either before inhalation of AA vapor or with concentrations of 5 and 10 mg/m^3.

In tests 3, 4 and 5 (15–26 mg/m^3) with maximum exercise load during the stepped test on the cycle ergometer, there was significant (15–25%) increase in cardiac output in all of the subjects. Maximum 0_2 uptake decreased by 26–28.5% at these concentrations. The decline in work capacity was related to diminished efficiency of cardiac function.

During the tests, we failed to demonstrate reliable changes in composition of peripheral blood under the effect of AA vapor. In test 3, insignifiction leukocytosis was observed in all subjects after 14 days of exposure, but in test 4, with the same concentration, this effect did not recur.

There was no impairment of heat-regulating mechanisms when temperature in the chamber was raised (tests 4 and 5).

In test 3 (15 mg\m^3) there was a decrease in mental mobilization, 15–25% increase in visual-motor response time, decrease in productivity of logical mathematical performance and significant decline of attention. In tests 4 and 5, elevation of temperature during exposure to AA vapor led to greater worsening of parameters than under optimum microclimate conditions.

Raising cabin temperature led to significant reduction in speed of dark adaptation: object detection time showed a 1.5–2.5-fold increase. AA vapor affected light sensitivity only in a concentration of 26 mg/m^3, and adaptation time decreased by 20–50% in all of the subjects. With concurrrent exposure to AA vapor in this concentration and elevated temperature, dark adaptation time reverted to the baseline.

Rate of glycolysis in erythrocytes decreased by 20–30%, while 2,3-diphosphoglycerate content decreased by 30–40% after 24 h of exposure to concentrations of 15 and 26 mg/m^3 (tests 3, 4 and 5). These changes persisted throughout the exposure period.

The immunity system did not change during the tests. Allergological tests revealed absence of sensitization to AA. Acid=-base equilibrium remained stable in all of the studies.

In the control experiment, ethylene content of exhaled air increased by 2–3 times during the heat test. Inhalation of acetic acid vapor in a concentration of 15 and 26 mg/m^3 led to 3- and 6-fold increase, respectively, in ethylene concentration. The combined effect of AA vapor and high temperature caused a 7–12- and 15–20-fold increase, respectively, in ethylene content, as well as 2–3-fold increase in pentane content [15].

All of these changes in functional parameters with use of 15 and 26 mg/m^3 AA appeared 3–5 days after start of exposure and persisted throughout the period of AA inhalation.

There are some difficulties involved in interpreting the demonstrated changes, since they can be evaluated both as a manifestation of adaptation to the factor in question and as the result of the adverse deleterious effect of AA vapor. Regulation of levels of deleterious impurities depends on the characteristics of functional changes that occur in the course of a test.

Reversible adaptive changes in functional parameters can be considered permissible [1] if such changes do not affect health or work capacity.

The distinction of toxicometric tests on man is that only a dosage (concentration) that equals or exceeds insignificantly threshold levels can be used. High doses cannot be tested for ethical reasons. For this reason, in studies where volunteers participate it is difficult to determine the difference between processes of adaptation and compensation of the effects of pathogenic factors, and we cannot track the disruption of these processes when dosage is increased. Accordingly, it is difficult to separate functional changes into adaptive and deleterious ones.

The minimal efffective concentration is a labile entity and depends on many factors. For this reason, use of the threshold concentration we found for validation of MPC is legitimate only under the relatively optimum living conditions described above.

Our findings warrant the belief that AA in concentrations of 15 mg/m^3 or more elicits the following adverse changes: decline of physical work capacity for maximum loads, intensification of lipid peroxidation (according to increase in hydrocarbon concentration in exhaled air), worsening of psychophysiological parameters and slower dark adaptation.

Change in erythrocyte metabolism are apparently an indiction of adaptation to the factor in question, since the parameters remained within the physiological range.

There was no appreciable worsening of physiological parameters during exposure to AA in a concentration of 15 mg/m^3, which is indicative of occurrence of adaptation to this factor. At the same time, adaptation is not complete, since it does not lead to return of functional parameters to the baseline level.

Thus, prolonged exposure (up to 15 days) to AA vapor in concentrations of 5 and 10 mg/m^3 does not have a noticeable effect on man in a closed chamber. Under the same conditions, a concentration of 15 mg/m^3 was found to be the minimal effective (threshold) dosage.

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CARDIAC RHYTHM OF ANIMALS CONSUMING RECLAIMED WATER DIFFERING IN CONCENTRATION OF SODIUM AND POTASSIUM IONS

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 22, No 1, Jan–Feb 88 (manuscript received 24 Sep 86) pp 61–63

[Article by V. A. Kondratyuk and M. S. Gnatyuk]

[English abstract from source] The effect of reclaimed potable water on cardiac rhythms of 190 noninbred white male rats was investigated in a 6-month experiment. The water contained 25.0 to 100.0 mg/l sodium and/or 2.5 to 10.0 mg/l potassium. The water containing 100 mg/l sodium and 10 mg/l potassium caused changes in both compartments of the autonomic nervous system controlling cardiac rhythms. The water containing 75.0 and 50.0 mg/l sodium and 7.5 and 5.0 mg/l potassium produced insignificant changes in cardiac rhythms. The water containing lower concentrations of sodium (25.0 mg/l) and potassium (2.5 mg/l) had no effect.

[Text] At the present time, mathematical methods of analysis of cardiac rhythm, which yield a quantitative and qualitative evaluation of the circulation-regulating system in the presence of variuos endogenous and exogenous factors, are used extensively in studies of the cardiovascular system [1–3, 6]. An important role in maintaining homeostasis is attributed to such ions as sodium and potassium. Thus, increase in sodium concentration in drinking water is the cause of hypertension in man, sodium and potassium are involved in maintaining normal myocardial function [7, 9]. Various endogenous and exogenous factors can alter appreciable the concentration of sodium and potassium in the body, causing change in cardiac function [4]. Our objective here was to investigate cardiac rhythm disturbances in animals consuming water with different concentrations of sodium and potassium.

Methods

We conducted a 6-month experiment on 190 mongrel male white rats initially weighing 120–140 g. The animals were kept under vivarium conditions on the regular diet, which differed only in quality of water. The control (1st) group of animals were given dechlorinated tap water of the hydrocarbonate-calcium type containing 24–33 mg/*l* sodium, 3.2–4 mg/*l* potassium and total mineral content of about 500 mg/*l*. The water for experimental groups of animals was based on reclaimed water that was subsequently upgraded. We used ion-exchange resins for this purpose (KU-2-12 p.ch. [пч—maximum or limited purity?], AV-17-8 p.ch.; PAU, MP-16, SP-6). The quality of

the drinking water conformed to GOST [State Standard] 2874-73 specifications (Table 1). The 2d group of animals was given reclaimed water, the 3d—water containing 10 mg/l potassium, the 4th—water with 25.0 and 2.5 mg/l sodium and potassium. The water consumed by the 5th group of animals contained 100 mg/l sodium and 10 mg/l potassium, the 6th—75.0 and 7.5 mg/l, respectively, the 7th—50 and 5 mg/$.l$, and the 8th—25 and 2.5 mg/l. The water was provided in automatic feeding bottles.

Table 1. Physicochemical properties of reclaimed potable water

Parameter	Measurement units	Quantitative expression
Odor	Score	0
Flavor	"	0
Transparency	cm	30
Color	—	Colorless
pH	—	7—3
Hardness	mg·eq/liter	1.0—1.2
Calcium	mg/liter	14.0—16.0
Magnesium	mg/liter	3.6—5.4
Sodium	mg/liter	—
Potassium	mg/liter	—
Chlorides	mg/liter	11.0—16.0
Sulfates	mg/liter	18.0—16.0
Alkalinity	mg/liter	30.5—38.0
Dry residue	mg/liter	100

In the study of cardiovascular system function, we measured the animals' arterial pressure using a plethysmometric unit [5], and recorded electrocardiograms (ECG) on a 16-channel encephalograph at a tape feeding rate of 100 mm/s.

In each case, we analyzed at least 100 R—R intervals on the ECG, and we plotted variation pulsograms. In accordance with data in the literature, we examined the following parameters of cardiac rhythm: mathematical expectation (mean)—M, standard deviation (σ), asymmetry (As), excess (Ex), mode (MO), mode amplitude (AMO), variational spread (ΔX), coefficient of variation (V), index of regulatory system stress (IS) and autonomic rhythm indicator (ARI) [1, 2]. We also determined heart rate (HR) and BP. The digital data were submitted to statistical processing on an Elektronika BZ-21 microcalculator using a program of parameters of normal distribution of a sample [8], and they are listed in Table 2.

Results and Discussion

Investigation of heart rhythm parameters of rats revealed (Table 2) that maximum changes were in the 3d, 4th and 5th experimental groups. In these cases, there was change in virtually all of the tested parameters of cardiac rhythm. We found that M and MO rose and HR decreased with chronic exposure to sodium and potasisum, which is indicative of increased tonus of centers of parasyumpathetic innervation of the heart [2]. The increase in these groups of variation spread (ΔX) confirms the presence of vagal hypertonus. BP was significantly diminished in the 4th and 5th groups of animals; in the 3d group it did not differ appreciably from values for the 1st and 2d groups. It should be noted that the changes in the above-mentioned parameters were less marked in the 6th group, whereas in the 7th and 8th groups they either differed little or were the same as in control animals. We see from these

findings that the heart, as a sensitive indicator of various factors, reacts and changes to a new functional level, although not too significantly, with long-term intake by animals of water containing 100 mg/*l* sodium, 10 mg*l* potassium and both sodium and potassium in concentrations of 100 and 10 mg/*l*, respectively.

Table 2. Parameters of mathematical analysis of cardiac rhythm of rats under the effect of sodium and potassium ions

Parameter	Group of animals							
	1	2	3	4	5	6	7	8
M, arbitr. units	109	108,7	118,3	115,2	115,4	114,7	113,6	108,4
σ	4,2	4,3	4,8	4,23	2,5	1,56	4,5	3,8
As	−0,089± ±0,12	−0,092± ±0,15	−0,101± ±0,228	0,3238± ±0,24	0,252± ±0,24	0,110± ±0,026	−0,105± ±0,18	−0,091± ±0,17
Ex	−2,569± ±0,21	−2,44± ±0,28	−1,519± ±0,45	−2,111± ±0,42	−1,894± ±0,48	−2,38± ±0,42	−2,46± ±0,33	−2,54± ±0,45
MO	104,0	112,0	120,0	120,0	115	115,2	112,8	106,2
AMO, %	12	15	20	20	18	12	10	10
ΔX, arb. units	14,4	14,3	21,6	20,0	20	20,8	20	15,2
V, %	25,9	25,2	27,3	27,2	46,1	30,2	25,2	27,8
IS, arb. un.	60	46	38	31	36	34	51	54
ARI, " "	66	62	41	42	43	42	44	62
BP, mm water	70,2±3,3	68,4±5,6	73,5±3,9	49,8±6,6	52,1±4,3	54,3±3,9	64,2±3,9	68,5±4,6
HR/min	450,8±15.3	452,5±18,2	417,4±12,9	426,9±9,3	423,5±12,1	428,5±15,2	442,6±16,1	453,5±15,3

The dynamics of asymmetry and excess in these cases were indicative of appreciable activity of transitional processes, which were not obsrved in animals consuming water with lower concentrations of sodium and potasisum. The slight change in As and Ex in the 7th and 8th groups of animals is indicative of stability of regulatory systems, i.e., absence of active transitional processes.

At the same time, it must be noted that increase in AMO, as well as the insignificant decline of IS that occurred in the 3d, 4th and 5th groups of animals, are indicative of stress in the sympathetic branch of the autonomic nervous system [2].

On the basis of our results, it can be concluded that we demonstrated signs of dysregulation in the form of rhythm slowing in the presence of some intensification in activity of the sympathetic nervous system in the 3d, 4th and 5th groups of rats, which presented the most marked changes in parameters of mathematical analysis of heart rhythm. It should be noted that we did not encounter a breakdown of adaptation, which is characterized by centralization of cardiac rhythm control with increase in pulse rate and marked increase in sympathetic nervous system activity, in rats of the 3d, 4th and 5th groups.

It can be concluded on the basis of the foregoing that chronic intake by rats of reclaimed potable water with 100 mg/*l* sodium and 10 mg/*l* potassium, either individually or together, has an adverse effect on the circulatory system, as indicated by the demonstrated signs of dysregulation and stress in both branches of the autonomic nervous system that control cardiac function.

There were insignificant changes in parameters of mathematical analysis of cardiac rhythm in the 6th and 7th groups of aniamls, which consumed water with 75 mg/*l* sodium and 7.5 mg/*l* potassium, as well as 50 and 5 mg/*l*, respectively, and they were indicative of satisfactory adaptation of the body to this situation.

The absence of appreciable deviations of parameters of mathematical analysis of cardiac rhythm in rats given water with 25 mg/*l* sodium and 2.5 mg/*l* potassium (8th group) indicates that these concentrations are harmless to the animals.

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VALIDATION OF MAXIMUM PERMISSIBLE CONCENTRATION OF UREA IN RECLAIMED POTABLE WATER AND EVALUATION OF ITS BIOLOGICAL EFFECT

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 22, No 1, Jan–Feb 88 (manuscript received 6 Jan 86) pp 63–66

[Article by N. V. Mironets, R. V. Savina, I. S. Kucherov, V. V. Solntseva and N. V. Martyshchenko]

[English abstract from source] The purpose of the study was to identify maximum allowable concentrations of urea in reclaimed potable water. The urea concentration equal to 80 mg/l is the threshold dose influencing the taste and flavor of water. Urea is a low toxicity substance (LD_{50}=14,300 mg/kg), the effect of which is not cumulative. However, when used in high doses it affects bioenergetic and cholinergi processess and causes changes in ECG, higher nervous activity and visceral structure. It was been shown that when applied to warm-blooded animals the acting dose of urea is 14.3 and 1.43 mg/kg (1/10000 and 1/100000 LD_{50}), the threshold dose is 0.72 mg/kg (1/20000 LD_{50}), and the ineffective dose is 0.36 mg/kg (1/40000 LD_{50}) which amounts to the concentration of 10 mg/*l*. In terms of toxic effects the dose equal to 10 mg/*l* is taken to be the maximally allowable concentration of urea. It is recommended to use the Laham biotest for measuring urea in water.

[Text] At the present time, water reclaimed in various regeneration systems is often used in manned closed environments. Recovery of water in reclamation systems may be complicated by penetration of urea into it, which is the main constitutent of the initial product [1–5, 6, 8–10], which may have an adverse effect on water quality and on man.

There is a need to regulate urea content of reclaimed water and determination of the mechanism of its effect on warm-blooded animals. We are offering here an experimental validation of the maximum permissible concentration (MPC) of urea in reclaimed water.

Methods

We used the method of S. N. Cherkinskiy and G. N. Krasovskiy, which they proposed for setting MPC of toxic substances in water reservoirs, to set the standard for reclaimed potable water.

When calculating the minimal ineffective dose (MID), we considered the mean daily water intake to be 2.5 *l* and that average weight of an individual is 70 kg.

Our work consisted of the following stages: 1) investigation of the effect of urea on organoleptic properties of water; 2) investigtion of the nature and degree of biological action of urea in acute, subacute and chronic experiments, which determination of effective, threshold doses and MID; 3) determination of MPC of urea in reclaimed potable water.

The effect of urea on organoleptic properties of water was investigated by the team method and it was given a grade on a 5-point system at a temperature of 20°C. The results were submitted to statistical processsing with determination of the bottom confidence limit. Determination of maximum parameters of toxicity was made using 50 white rats (males) by the method of Deichmann and LeBlanc. Species-specific sensitivity was determined on guinea pigs, white mice and rabbits (10 animals of each species).

The nature and extent of biological action of urea were investigated in a subacute experiment (30 days) on white rats given urea in doses of $^1/_{50}$ and $^1/_{20}$ of LD_{50}. For determination of urea MID, we conducted a 6-month chronic sanitary-toxicological experiment, in which we tested doses of 14.3, 1.43, 0.72 and 0.36 mg/kg ($^1/_{1000}$, ($^1/_{1000}$, $^1/_{10,000}$, $^1/_{20,000}$, $^1/_{40,000}$ of LD_{50}). In selecting urea dosage, we were governed by data in the literature [1–3], the results of testing organoleptic properties of water, as well as those from the acute and subacute experiments. In the sanitary-toxicological experiment, we used a set of physiological biochemical, hematological and other parameters that enabled us to determine the biological effect of urea.

We tested mongrel white rats (60 specimens) in the chronic experiment.

In addition, we had to investigate the effect of urea on the central nervous system, for which purpose we used conditioned reflex methods and investigation of threshold-summation index (TSI). To assess the cardiovascular system, we used a functional hypoxia in an altitude chamber. We tested the activity of several enzymes: blood serum cholinesterase (CE), asparate and alaklnine aminotransferases (AsAT and AlAT). We measured serum urea content as a specific parameter. Basal metabolism was examined.

To monitor urea content of reclaimed water, we used a biological test for urea. The method is based on the principle that urea, in the presence of Fe^{3+} semicarbazide in a markedly acid medium, a red complex with diacetyl monoxide, which is submitted to photometry on a photoelectrocolorimeter; 1 m*l* ethanol equals 0.01 mg/m*l* urea. The concentration of urea (X), in millimols/liter sample, is calculated from the obtained optical density of the sample (A) and ethanol (B) using the following formula:

$$X = \frac{A}{B} 10 \text{ mg/}l$$

Results and Discussion

As a result of the experiments, it was established that urea, when added to water in different concentrations, does not alter its transparency or odor, but does impart a slightly bitter taste to the water. The bottom of the confidence limit of perception

of an aftertaste graded at 1 (96±7.7) in strength is at the level of 80 mg/*l*, whereas with a rating of 2 (160±8.0) it is at 144 mg/*l*.

It was established that LD_{50} of urea for white rats is 14,300 mg/kg. Median lethal dose for white mice is 18,000 mg/kg. Thus, urea is among substances with low toxicity. It does not have cumulative properties.

Dynamics of biochemical parameters of rats given urea in the chronic experiment

Parameter	Time of test, months	Urea dosage, mg/kg				Control 1	Control 2
		14,3	1,43	0,72	0,36		
CE	BL†	0,65±0,045	0,63±0,076	0,60±0,045	0,64±0,050	0,62±0,051	0,60±0,045
Urea	BL	6,43±0,972	6,51±1,007	6,30±0,340	6,40±1,010	6,23±0,251	6,04±0,350
CE	1	0,35±0,033*	0,50±0,039	—	—	0,58±0,030	—
Urea	1	10,6±1,327*	7,59±1,234	—	—	4,96±0,494	—
AsAT	1	0,57±0,029*	0,23±0,016	—	—	0,21±0,015	—
AlAT	1	0,34±0,022*	0,16±0,023	—	—	0,16±0,020	—
CE	2	0,46±0,060	0,55±0,050	0,65±0,020	0,63±0,030	0,60±0,080	0,60±0,045
Urea	2	11,6±1,106*	8,16±0,375	5,49±0,350	4,91±0,350	5,46±0,374	5,61±1,250
AsAT	2	0,43±0,013*	0,21±0,034	0,54±0,010	0,52±0,030	0,20±0,029	0,53±0,020
AlAT	2	0,31±0,021*	0,15±0,013	0,48±0,001	0,49±0,030	0,14±0,010	0,51±0,020
CE	3	—	—	—	—	—	—
Urea	3	9,68±1,159	7,49±0,554	—	—	6,36±0,351	—
AsAT	3	0,19±0,049	0,24±0,039	—	—	0,16±0,074	—
AlAT	3	0,12±0,033	0,19±0,030	—	—	0,12±0,010	—
CE	4	0,46±0,040*	0,48±0,020*	0,59±0,020	0,52±0,007	0,57±0,030	0,54±0,002
Urea	4	8,42±0,303	7,56±0,403	6,61±0,300	6,33±0,400	8,09±0,403	6,04±0,360
AsAT	4	0,37±0,033	0,32±0,033	0,52±0,001	0,52±0,007	0,34±0,042	0,52±0,003
AlAT	4	0,23±0,023	0,26±0,012	0,51±0,003	0,51±0,003	0,27±0,039	0,51±0,001
Ce	6	0,85±0,050*	0,60±0,070	0,56±0,015	0,58±0,015	0,50±0,030	0,57±0,030
Urea	6	8,34±0,756	7,23±0,286	6,58±0,300	7,28±0,300	7,61±1,208	7,07±0,960
AsAT	6	0,22±0,024	0,29±0,099	0,52±0,006	0,52±0,006	0,31±0,039	0,52±0,006
AlAT	6	0,22±0,018	0,20±0,049	0,50±0,003	0,51±0,006	0,27±0,042	0,51±0,001

* $p<00,5$ †Baseline

As a result of giving animals urea in doses of $^1/_5$ and $^1/_{20}$ of LD_{50}, we demonstrated a reliable increase in serum urea content, decrease in hemoglobin, increase in leukocytes and appreciable, reliable changes in parameters characterizing the animals' higher nervous activity. In both experimental groups, an elementary defense reflex was developed in only 44.4% of the animals, versus 92.5% in the control group. The histological structure of internal organs (neurons, liver, kidneys, intestine, etc.) was also altered.

We tested the following doses of urea in the chronic experiment: 14.3, 1.43, 0.72 and 0.36 mg/kg ($^1/_{10,000}$, $^1/_{20,000}$, $^1/_{40,000}$ of LD_{50}).

As a result of these investigations (see Table), it was established that there is a decrease in serum CE content and increase in serum urea under the effect of urea in a dosage of 14.3 mg/kg. In addition, we observed increase in activity of AlAT and

AsAT, changes on the ECG and in morphological parameters of blood. With use of urea in a dosage of 1.43 mg/kg, we observed a decline in CE activity and hematological changes; with use of 0.72 mg/kg, there was a one-time decrease in erythrocytes and change in basal metabolism. A dosage of 0.36 mg/kg urea did not elicit any changes in experimental animals.

Thus, the biological action of urea consists of the following: it has a stimulating effect on processes of protein biosynthesis, since serum urea content is elevated, it impairs cholinergic nerve elements, as indicated by changes in CE activity, it affects the adaptability of the heart (ECG changes) and higher nervous activity.

It should be stressed that there is particularly distinct demonstrattion of the mechanism of action of urea on energy metabolism. Stimulation of protein metabolism under the effect of urea in the first few months of the experiment causes elevation of level of energetic processes. Subsequently, they decline, which is apparently attributable to the body's inability to maintain metabolism on a high level for a long period of time, as well as impairment of its compensatory potential.

Thus, in validating the MPC of urea in reclaimed potable water, it was established that 14.3 and 1.43 mg/kg ($^1/_{1000}$ and $^1/_{10,000}$ of LD_{50}) are effective doses of urea, the threshold dosage is 0.72 mg/kg ($^1/_{20,000}$ of LD_{50}) and MID is 0.36 mg/kg ($^1/_{40,000}$ of LD_{50}).

On the basis of comparison of threshold concentration for organoleptic properties (80 mg/l) to MID scaled to concentration (10 mg/l), for a person weighing 70 kg and consuming 2.5 l, a concentration of 10.0 mg/l according to toxicological harmfulness is recommended as the MPC for urea content in reclaimed potable water. This concentration coincides with the MPC for urea in water of reservoirs [7].

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BIOLOGICAL PATTERNS OF GROWTH IN POSTNATAL ONTOGENESIS OF LOWER PRIMATES

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 22, No 1, Jan–Feb 88 (manuscript received 22 Apr 87) pp 66–70

[Article by Yu. N. Kurochkin and G. S. Belkaniya]

[English abstract from source] In 840 male rhesus-monkeys relationships between age, height, weight and growth rate were examined. In terms of growth rate the following five age periods were identified in the predefinitive stage of postnatal ontogenesis: childhood—from birth to 9 months of age, adolescence—from 9 months to 3 years, accelerated growth or pubescence—from 3 to 4.5 years, growth completion—from 4.5 to 7–8 years, and physiological maturity (definitive stage)—over 8 years of age. The above age periods derived from growth curves are consistent with the development of the dental system, reproductive organs and other biological signs of postnatal ontogenesis. The relationships between calendar age, height and weight with respect to each age period are described by linear regression equations. The basic patterns of physical development, period of postnatal ontogenesis and somatometric characterization described above help to objectively monitor the physical fitness of rhesus-monkeys, to adquately select animals identical in terms of their biological age, and to reliably plan long-term studies on this primate species.

[Text] Questions of growth and physical development of various representatives of the mammalian class hold an important place in current biomedical research, and they are drawing the attention of specialists in different biological sciences (anthropology, morphology, age-related physology). Growth patterns have been studied the most in man and laboratory animals (rats, rabbits). Some distinctions referable to growth and physical development have been demonstrated in primates [5, 6, 10]. The philogenetical similarity of man and other primates is the reason for interest in ontogenesis of lower primates [11, 14].

Three stages are distinguished in physical development of mammals: predefinitive, definitive and postdefinitive. The expediency of distinguishing these stages is validated the most in studies of patterns of human ontogenesis [8]; however, at the present time, it is virtually not used to describe ontogenetic development of animals. Studies of the dynamics of physical development of man have shown that, in order to determine

the stage and period of ontogenesis, it is necessary to obtain an integral description of biological age according to degree of development of somatic and functional signs [2]. The rate of such development and duration of relevant age periods differ, not only in different animal species, but in different individuals of the same species and chronological age. For this reason, assessment of biological age of an animal in contemporary biomedical studies is the deciding prerequisite for identifiction of homogeneous groups of animals. At the same time, while growth processes present marked dynamics, investigation of patterns of physical development at the predefinitive stage of ontogensis acquires importance.

The increasing use of primates in special experiments, including the biomedical program of space research, makes it necessary not only to have general biometric data, but age-related characteristics. However, the available information concerning physical development of primates on the basis of dynamics of body growth is quite incomplete and fragmentary [7, 13]. The first experience in preparing primates for a biological experiment aboard a specialized artificial earth satellite of the Cosmos series revealed that methods of selecting and examining animals were used empirically to a significant extent. The main reason for this was absence of necessary information about developmental biology of primates due to the medical-technical restrictions of the experiment. The absence of somatometric data on dynamics of physical development of Macaca rhesus monkeys, needed to predict changes in the main limiting parameters (height, weight) in the course of the rather lengthy preparatory stage of the experiment, made it difficult to screen and prepare the flight group of monkeys. Aside from their applied relevance, comparative studies of growth and development patterns of somatic and functional features of biological age make it possible to establish comparability of periods of ontogenetic development, which is a mandatory prerequisite for validation of feasibility of extrapolation to man of experimental data obtained on monkeys. The need for such validation is determined by the marked age-related differences in reactivity and resistance of the main physiological systems.

Our objective here was to investigate the biological patterns of growth in postnatal ontogenesis of lower primates, as well as to elaborate a method of somatometric monitoring of the growth process and predicting growth in planning long-term experiments.

Methods

The studies were pursued on 840 male Macaca rhesus (M. mulatta) monkeys 3 days to 14 years old. In this work, we used the results of measuring weight and body length, as well as data characterizing development of the dental system and testes. The animals were weighed on a medical scale in the morning, before feeding. Body length (arbitrary height in seated position corresponding to the monkey's position in the immobilization device of the biomodule capsule) was measured under ketamine anesthesia using Martin's sliding calipers. The animal was placed on a firm even surface on its left side, the lower extremities were flexed at the hip and knee joints and brought slightly up toward the trunk. Concurrently, the upper extremities were placed perpendicularly to the trunk. The head was immobilized in such a way that the plane traversing the incisors and occipital tubercle would be perpendicular to the body's long axis. Measurements were taken between the top point of the sinciput and the inferior surface of the ischial tuber. Development of the dental system was evaluated visually according to the number of erupted deciduous and permanent

teeth. Longitudinal and transverse (length, width) dimensions of the testes were measured with medical sliding calipers.

We studied dynamics of monkey growth by the method of horizontal examination of a sample [10]. Measurement data were grouped according to age at 3-month intervals and processed on an Iskra-260 minicomputer using a program that was developed. We studied the relationship between absolute age, height and weight, as well as derivative parameters: weight-height ratio and specific rate of growth and weight gain. Derivative parameters were calculated using conventional formulas [9, 11]. The dynamics of growth were compared to extent of development of the dental system, dimensions of testes and intensity of secondary sex characters.

Results and Discussion

The dynamics of growth throughout the entire postnatal period of ontogenesis are characterized by differences in intensity (Figure 1). Maximum growth rate is observed in the period from birth to 9 months of age. Thereafter, growth slows down significantly and holds at a relatively constant (with minor fluctuations) level to the age of 3 years. In the periods of 3–5 years, growth rate increases rather markedly (pubertal jump) and is followed by slowing, and by the age of 7–8 years growth in length is essentially completed in male Macaca rhesus monkeys. It is important to mention that these distinctions are demonstrable for all of the parameters of physical development (height and weight, weight-height index) used for analysis. Thus, according to specific growth rate in the course of postnatal ontogenesis of male Macaca rhesus, the following age periods can be distinguished, which have different growth rates and biological patterns (see Figure 1): I—from birth to 9 months (childhood); II—from 9 months to 3 years (adolescence); III—from 3 to 4.5 years (period of accelerated growth, or pubertal period); IV—from 4.5 to 7–8 years (period of completion of growth process; V—over 8 years (period of physiological maturity of the monkeys).

The periods distinguished according to general parameters of physical development (height and weight, weight-height ratio) conform well to the pattern of tooth development (Figure 2) and dynamics of testicular growth. Thus, growth of deciduous teeth is completed by the age of 7 months, i.e., in childhood. Replacement of deciduous teeth with permanent ones starts at 28 months (in adolescence) and ends at the age of 5.5 years (puberty). It should also be noted that it is expressly in the period of accelerated growth (age period III) that most deciduous teeth are replaced with permanent ones. Last to erupt are the canines, which reach their final size by the age of 6–7 years in male Macaca rhesus, i.e., in the period of completion of growth processes. The pattern of development of the testes is characterized by a genital type of growth. Testicular growth in periods I and II is very slow, and it is only at the age of 40–42 months (period III) that growth rate increases appreciably, reaching a maximum at 5–6 years of age, in the period of completion of growth processes. The testes attain their definitive dimensions by the age of 7–8 years.

With reference to the general biological description of postnatal development of the monkeys, we can mention the following. In period I, the monkeys learn the basic forms of locomotor behavior, they are weaned and switch to independent feeding. Their mothers then become pregnant and, according to the adopted technology of raising monkeys in captivity, offspring are removed at the age of 8–10 months. In period II, there is completion of all forms of locomotor behavior, permanent teeth begin to grow

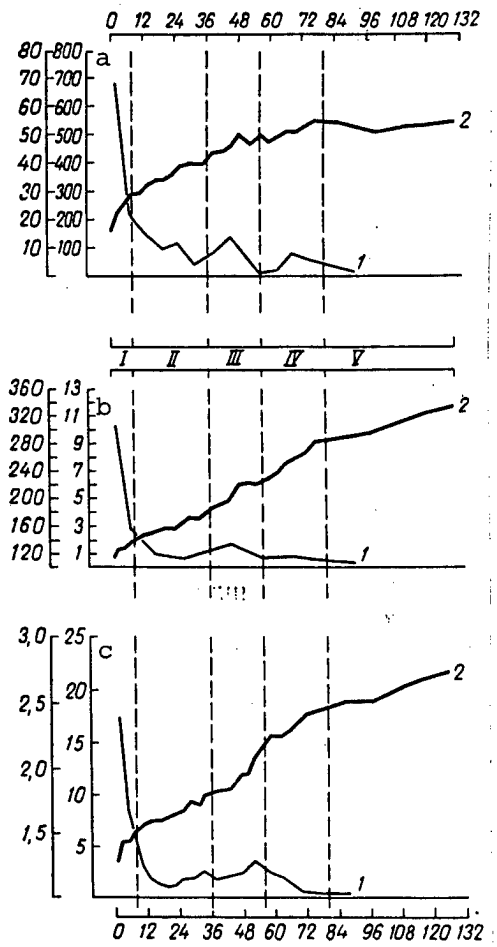

Figure 1.
Dynamics of postnatal growth of male Macaca rhesus monkey
a) height (mm), weight (kg), weight–height index (arbitrary units), specific rate of parameter (SR)
X-axis—animals' age (months), y-axis: a, b, c—left: specific growth rate (SGR); right: height (h, mm), weight (m, kg) and weight-height index (WHI) (arbitrary units), respectively. Here and in Figure 2, I–V refer to age groups.
1) SGR
2) in (a)—height, in (b)—weight and (c)—weight-height index

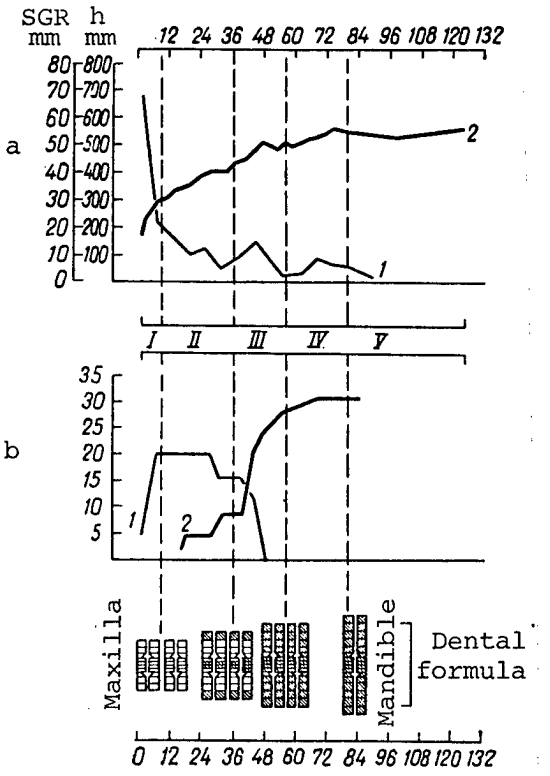

Figure 2.
Tooth development in postnatal ontogenesis in male Macaca rhesus monkeys. Height (in mm) and specific growth rate (SGR); dynamics of replacement of deciduous teeth (DT) by permanent teeth (PT); dental formula at start and end of main periods of physical development
X-axis—animals' age (months); y-axis:
a) left—SGR, right—height (h, mm)
b) number of teeth
c) dental formula
1) deciduous teeth
2) permanent teeth

and replace the deciduous ones. In period III, the monkeys enter into a phase of accelerated growth and physical development of reproductive organs, there is differentiation of sex in secondary sex characters and formation of sexual dimorphism characters. Group behavior of the animals is formed in accordance with the degree of physiological maturity. In period IV, there is completion of processes of growth and physical development of the animals, and formation of somatic types. By the end of this period, the reproductive system is functionally mature.

It is important to stress that the dynamics of body growth are very similar in male Macaca rhesus monkeys and man, and although the growth process extends over a longer period in man, the periods distinguished in monkeys according to the main patterns of growth and physical development are comparable to the periodization adopted for man [1, 14]. In other words, growth and physical development of lower primates are naturally and philogenetically the closest model of growth and physical development of man.

Table 1.
Coefficients of paired and multiple correlation between age, height and weight of male Macaca rhesus in four age periods

Parameter	Period			
	I	II	III	IV
Age--body length	0,8	0,7	0,7	0,7
Age--weight	0,8	0,7	0,7	0,8
Age--length--weight	0,9	0,7	0,7	0,8

Note: Levels of significance of all coefficients of correlation correspond to $p<0.001$.

In view of the appreciable qualitative and quantitative differences in physical development of monkeys at different ontogenetic stages and uneven intensity of the growth process, we submitted the data to statistical processing (including regression analysis) separately for each of the four age periods. It should be noted that the mathematical description of the entire curve of growth dynamics formalizes the process to a significant extent and leads it away from its biological essence [2, 12]. In addition, the high degree of correlation between height, weight and chronological age (Table 1) enabled us to describe the relationship between age, height and weight for each distinguished period quite accurately with equations of linear regression. The obtained equations, along with the other characteristics (Table 2), enable us to determine biological age from the height and weight measurements, to assess with aknown chronological age the degree of conformity of physical development of the monkeys to their age in any of the four periods, to predict the probable increase in height and weight within the current and subsequent age periods.

Table 2. Biological characteristics of periods of postnatal ontogenesis in male Macaca rhesus monkeys

Parameters	Predefinitive stage				Definitive stage (start)
	I	II	III	IV	
Chronological age, months	0,1—9	>9—36	>36—54	>54—96	>96
Body length (h), mm	160—283	300—400	410—470	480—550	>550
(BA = $bh - a$)	0,07p—8,5	0,12p—19	0,15p—24	0,5p—170	—
Weight (m), kg	0,45—1,75	2,1—3,6	3,9—6,0	6,3—9,5	>9,5
(BA = $bm + a$)	4,0м+4,4	5,2м+9,3	3,3м+27,0	8,6м+6,5	—
Number of deciduous teeth	2—20	20—16	16—0	0	0
Number of permanent teeth	0	0—8	8—28	28—32	32
Dimensions of testes, mm					
length	8—10	12—25	27—45	47—55	>55
width	6—7	9—16	18—29	31—35	>35

Notes: BA—equation of linear regression for determination of biological age.
Characteristics correspond to beginning and end of periods.

Thus, as a result of these studies we demonstrated the main patterns of physical development, we determined the chronological range of completion of the predefinitive and start of the definitive stage, and we distinguished between four periods of growth

in postnatal ontogenesis for male Macaca rhesus monkeys. Use of an integral somatometric standard characteristic (height and weight, weight-height index, dental formula, dimensions of testes) for the distinguished age periods enables us to objectively monitor physical development of the monkeys, to pursue purposeful screening of animals and make it possible to plan the stages of a long-term experiment. The rather distinct comparability of age periods in the pattern of growth for man and animals is indicative of philogenetic similarity of representatives of one order of primates according to one of the most important biological characteristics. The approaches we developed and data obtained on somatometric description of the dynamics of physical development of monkeys in postnatal ontogenesis constitute the basis for future studies to demonstrate the correlation between constitutional distinctions and functional reactivity of the principal physiological systems in different age periods under ordinary living conditions of animals and with exposure to diverse environmental factors.

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METHOD OF ASSESSING CHANGES IN BIORHYTHMOLOGICAL STRUCTURE OF HUMAN PHYSIOLOGICAL FUNCTIONS

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 22, No 1, Jan–Feb 88 (manuscript received 18 Aug 86) pp 71–73

[Article by I. F. Vaysburd]

[Text] Investigation of functional changes in man over a 24-h period is an important task for space medicine [1]. At the present time, there are several approaches to description of timing of human physiological functions and analysis of changes in them. Individual cosinor analysis (ICA), which involves creation of models of baseline data in the form of appropriate combinations of algebraic and trigonometric functions, has gained the greatest popularity [6, 7]. Complicated combinations and superpositions of the most elementary functions are used on large segments of cases in order to consider changes in level, acrophase and amplitude of biorhythm. This makes it difficult to compare models, interpret occurring changes and diminishes the prognostic value of the model.

Our method proposes analysis of variability of three parameters of a cosinor model (mean level, acrophase and amplitude) obtained for sliding observation intervals. The following objectives are set: to assess the degree of adaptation of the time structure of human physiological functions to extreme factors, to describe quantitatively the wandering zone not only of acrophases, but other biorhythm parameters, lability or stability of the circadian system [4]. The method is illustrated with the data obtained in the course of a study by B. S. Alyakrinskiy and S. I. Stepanova. This author expresses his profound appreciation to them for their consideration of this work.

Methods

We processed some data from a study on adpatation to inverted days, which was described in detail in the monograph by S. I. Stepanova [3]. The heart rate (HR) and sublingual temperature (T) were recorded every 2 h on 2 subjects, G. and K. At the start of the 10th day we performed a baseline examination. Then, after the subjects had stayed awake for 72 h, they changed to a sleep-waking schedule that was shifted by 12 h from the baseline. All of the clocks accessible to the subjects were also advanced 12 h. Testing under these conditions lasted 12 days. Both subjects were isolated from the outside world for all 25 days.

Mathematical processing of the obtained time series consisted of the following. Each stage (baseline and inverted) was divided into intersecting 72-h intevals with a 24-h

shift: 1st–3d, 2d–4th days, etc. At each interval, the baseline data were approximated with a function of the following appearance:

$$V_s(t) = M_s + A_s\cos\left[\frac{2\pi}{s}t - \varphi_s\right] + \epsilon_s(t) \qquad (1)$$

Using ICA with fixed s, determination was made of parameters of this model: M_s—mean level of physiological parameter, A_s—amplitude of its fluctuations and φ_s—time of attaining maximum value (acrophase). Remainders $\epsilon_s(t)$ were analyzed for normality and independence, after which we calculated appropriate statistics K_s [7], and using the F criterion we checked the zero hypothesis, $A_s=0$. These calculationss were made for each entire s in the interval of 10–36 h. From the obtained set of cosine curves we selected the function with $s=24$ h, if the corresponding P [2], $P_S<0.05$. Otherwise, we selected function $V_s(t)$ with the lowest P value provided $P_s>0.05$. If all values are $P_s\geq 0.05$ in some interval, we concluded that there was no reliable cosinor model in this interval. Such a system of chronobiological analysis had been discussed previously [5] for constructing cosinor models for large observation intervals.

Changes in parametres of model (1) in both subjects are illustrated in Figures 1 and 2. The baseline period was divided into 8 72-h intervals, and the inverted period into 10 such intervals. The values of parameters in each interval were determined using the chosen model $V_s(t)$.

The baseline wandering zone (BWZ) of biorhythm parameters, of course, refers to the range of changes in values of these parameters with variation of the interval in which model (1) is constructed. The table lists the range of BWZ of all parameters for both subjects.

Results and Discussion

Let us discuss the data for each subject separately, and then we shall compare them.

Subject G. In the baseline period, initial HR data were reliably close to 24-h models (i.e., S=24 h) in 6 out of 8 intervals. We chose models with $s=36$ h and $s=32$ h in the 1st and 6th intervals, respectively. The values for T were reliably approximated by 24-h models in all 8 baseline intervals. Staying awake for 3 days dramatically altered only HR amplitude, while the other parameters remained in the range of their BWZ. In this period, as well as the next 10 72-h intervals, baseline measurements of both HR and T were reliably simulated with function $V_s(t)$ with $s=24$ h. Analysis of variability of model (1) parameters during the period of inverted sleep-waking schedule shows that mean HR, HR acrophase and T exceeded the baseline wandering zones. As can be seen in Figure 1, the last parameter, T acrophase, entered into the BWZ in the interval of 9th–11th days in subject G. True, the amplitudes of HR and T exceeded the top of their BWZ in the last interval of the inversion period. This could have served as a cue to extend the observations in order to determine the stability of such changes, particularly since the mean level of both functions also rose in the same interval. At any rate, the values of all 6 analyzed parameters were within their BWZ in the interval of 9th–11th days, which could serve (considering the comment we made above) as grounds to maintain that the tested physiological functions of subject G. had adapted to the inverted sleep–waking schedule.

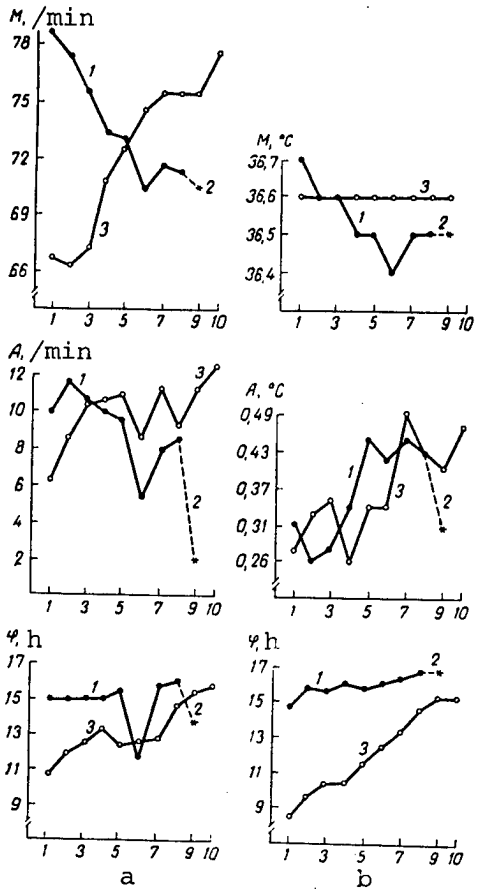

Figure 1.
Dynamics of biorhythm parameters of subject G. Here and in Figure 2:
a) HR b) T
X-axis—sequential number of 72-h interval
1) baseline changes
2) 72-h wakefulness
3) inverted sleep-waking period (acrophase shown also with 12-h shift)

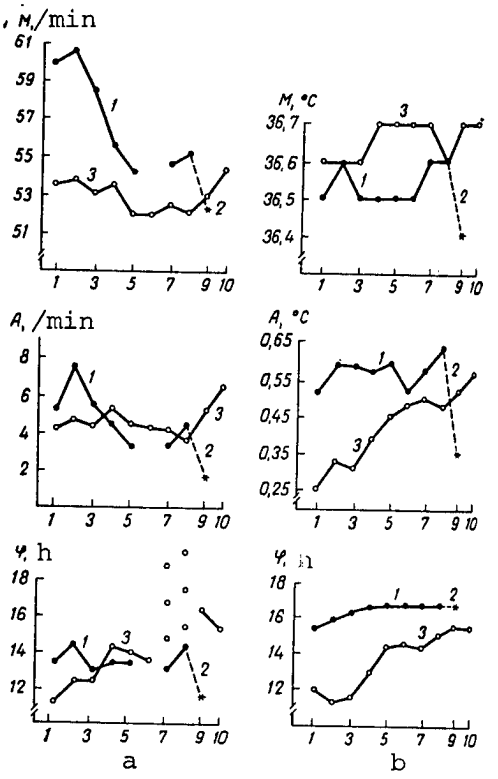

Figure 2.
Dynamics of biorhythm parameters of subject K.

Baseline zones of wandering of biorhythm parameters

Function	Parameter	Subject G.	Subject K.
HR	M/min	70.2—78.6	54.2—60.2
	A/min	5.4—11.6	3.3—7.4
	φ, h	11.8—16.0	12.9—14.4
T	M, °C	36.5—36.7	36.5—36.6
	A, °C	0.25—0.44	0.51—0.63
	φ, h	14.9—16.7	15.3—16.6

were approximated with a 26-h model.

Subject K. In the interval of 6th–8th days in the baseline studies, the initial HR of this subject was not modeled with a function like (1), whereas in the 7-9-day interval we selected the function, $V_s(t)$ with $s=36$ h. In all the other intervals, HR was reliably approximated with a 24-h model, and for T this model was reliable for all eight 3-day intervals of the baseline period.

Staying awake for 72 h led to exit from BWZ of all analyzed parameters with the exception of T acrophase [we chose $V_s(t)$ for this interval with $s=35$ h for HR and $s=24$ for T].

During the inversion period in the 7th and 8th 3-day intervals, baseline HR data As a result, in these intervals the HR acrophase assumed three different values (see Figure 2). HR, in the other intervals of this period, and T, in all 10 intervals, were approximated by 24-h models. Figure 2 shows that mean HR level, which dropped in relation to the BWZ, never did reach this zone in the 12 days of inverted schedule. HR acrophase and mean T exited from

their baseline zones in the last intervals. Thus, all of the parameters of rhythm of physiological functions of subject K. were never in their baseline zones simultaneously at any time over the 12-day period of inverted sleep–waking schedule. This warrrants the conclusion that subject K. did not adapt to the schedule change in 12 days.

Use of this method also permits evaluation of synchronization and desynchronization of the physoilogical functions studied. If we were to define desyncrhonization of two physiological functions as the difference between acrophases of these functions in the interval of interest to us, a simple calculation will show that, starting with the 5th –7th day of inversion, this distance is 1.1 h for subject K.. and 1.4 h for subject G., i.e., the time structure deviated less in G. from the initial synchronized state than it did in K.

In addition, this method allows us to compare the reaction of different parameters describing biorhythms of physiological functions. Finally, if we examine the diameters of BWZ, we could conclude that all BWZ diameters are larger in subject G. than the corresponding diameters in subject K. This warrants the conclusion that the circadian system is more constant in subject K.

Thus, use of model (1) for short sliding intervals covering all segments of observation and subsequent analysis of variability of its parameters in the baseline period, during exposure to extreme factors and in the adaptation period enables us to assess the degree of adaptation of physiologicall functions to factors and extent of their desynchronization under the effect of the factors, as well as to formalize some important concepts of biorhythmology.

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USE OF PRINCIPAL COMPONENT METHOD FOR ANALYSIS OF MULTIDIMENSIONAL QUANTITATIVE DATA IN BIOMEDICAL INVESTIGATIONS

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 22, No 1, Jan–Feb 88 (manuscript received 17 Feb 87) pp 73–75

[Article by S. L. Chekanova, T. M. Smirnova and M. A. Matrosova]

[Text] The principal component method (PCM) is being used with success for analysis of multidimensional biomedical data of the quantitative type. It permits compression of information contained in the measured parameters and concentration of its main part in several numbers, values of the first principal components (PC) that explain a significant share of the scatter of baseline data [1, 12]. PCM is used to solve three important classes of problems in the area of biomedical investigations: formation of general evaluations (integral parameters) on the basis of a set of observed characters; classification of objects of observation in the space of generalized parrameters; quantitative description of certain characteristics of objects as a function of values of integral ratings.

PCM involves the use of orthogonal conversion of observed variables in order to obtain new, uncorrelated variables—PC having the following properties: scatter of point projections over the first PC is at a maximum, as compared to all other directions; the sum of the squares of distances from original points to their projections on the firt PC is minimal.

Distinction of PC provides a system of integral parameters that are linear combinations of baseline characters and are subject to interpretation by physicians. The programs that are used for calculations by the PCM are part of the standard software of YeS computers [9, 10]. The data for computer calculations must be in the form of tables, the columns of which correspond to baseline parameters and the lines, to events. Using standard programs with the PCM, calculations are made in the following order: centering and standardization of baseline data; calculation of correlation matrix of baseline parameters; determination of factor structure of analyzed information [7]; calculation of PC for each object; calculation using the last two procedures after rotation.[1]

Rotation was used in order to simplify the factor structure of the data, and it involves organizing PC according to number of zero loads on them. Interpretation of components depends on the signs and values of coefficients in their expression as

[1]We used "varimax" rotation in the calculations.

baseline variables. In the definition of PC, characters with the greatest weight are retained, and all the others are not taken into consideration. It is convenient to list these characters in the table in order of diminishing informativeness [5, 7, 8].

Let us dwell briefly on the types of problems in biomedical research related to use of PCM. The first class of problems is related to formation of integral parameters (PC) and evaluation of tags in order to take the most informative[2] ones when studying dependence of functional load tests [4] and other factors [2], with which numerous parameters of the quantitative type are used. They could also include problems related to development and refinement of criteria for screening people to participate in spaceflights of different duration, formation of integral characteristics of work and rest schedules at different stages of long-term manned flights [6]. In the latter study, analysis was made of 24-h dynamics of integral parameters (first and second PC). In all of these problems, it is necessary first of all to single out several of the first PC that explain well the scatter of baseline information. Then, one must identify the informative parameters in these components, on the basis of which they were formed. The integral parameter—PC—is interpreted by physicians and is the basis for their conclusions [4]. The second class of problems is related to classification of objects or events (condition of an object at different points in time) in the PC space. These are problems of singling out homogeneous groups, as well as problems related to evaluation of the norm and its marginal variants [3]. The analyzed objects can be represented by points with identification numbers in the PC space. The groups in the PC space will help make proper determination of patterns related to tolerance to various factors. The third class of problems involves quantitative evaluation of characteristics of objects as a function of integral evaluations. It involves obtaining prognostic equations for resultant tags on the PC that characterize the factor structure of data referable to the factor in question. Ordinary regression analysis provides satisfactor results when there are independent or few mutually related tags (with addition of a new tag, the coefficients of regression change appreciably in size and even their sign could change). It is expedient to include the singled out PC in regression analysis as variables. This approach reduces their number in the regression equation and permits elimination of correlation between variables (PC are orthogonal), it reduces the influence of errors and simplifies evaluation of importance of variables. As a result, the regression equation will include parameters that have an appreciable effect on explained dispersion. When selecting PC, it is assumed that there is normal distribution of baseline parameters, although deviations from normal have little effect on the results of the procedure of singling out the PC. To obtain reliable results it is important to adhere to a certain correlation between quantity of variables analyzed and number of events [8].

In conclusion, let us discuss an example of using the PCM to compress data recorded in comprehenisve investigations. Data pertaining to changes in volume of body fluids and sodium balance obtained in a 120-day study with hypokinesia [11], as well as data pertaining to changes in balance of potassium, calcium, magnesium (data obtained by B. V. Morukov), fluid intake and diuresis (data of G. I. Kozyrevskaya) served as the object of joint analysis. There was a total of 12 baseline parameters and 5 PC providing for 88% overall dispersion. The procedure of varimax rotation led to distinction of a 5-dimensional basis, and 4 of the basis vectors coincided almost exactly with the 2d–5th PC. The first PC was represented by the weighted sum of all baseline

[2]Characters in which the greatest differences are demonstrable are considered informative. These tags should also have high coefficients of correlation with PC.

parameters with similar absolute values (from 0.13 to 0.42) for weight coefficients. This result is related to the choice of scale for baseline data (volume of liquid phases of fluid intake and diuresis was determined as percentage of corresponding baseline values, while electrolyte balance was expressed in milliequivalents per day of the study), with which the scatter was about the same for all baseline parameters. With varimax rotation, the PC with such complex structure was replaced with a vector, the maximum factor load of which provides for one of the baseline parameters—sodium balance. It was also found possible to single out a unique baseline parameter with maximum factor load. These parameters were (in order of diminishing intrinsic values of corresponding PC): volume of intracellular fluid, volume of extracellular fluid, calcium balance, volume of circulating plasma. Virtually all these parameters show little correlation with one another, while each of the baseline ones not included in this group had a rather high coefficient of correlation with one of the parameters of this group. Consequently, the original set of parameters can be reduced. Five parameters were the most informative. Thus, among the original parameters, there is distinction of a set of linearly independent sources of individual variability. Of course, independence of the basis, as well as causes of individual variability of corresponding parameters, require validation on the extrastatistical, meaningful level, i.e., in terms of the problem area in which the experiment was performed. For example, in the case in question, the results of using the PCM can be interpreted qualitatively. It can be assumed that the sources of individual variability of changes in fluid-mineral balance with use of antiorthostatic [head-down tilt] hypokinesia are attributable to dissimilar capacity to retain water in the vascular, interstitial and intracellular spaces, deposition of sodium, as well as dissimilar sensitivity of calcium metabolism on bones to decline of motor activity. Thus, in this case PCM also emerges in the role of the source of hypotheses for a physiological investigation, closing the chain of feedback between the experiment and statistical analysis of its results.

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UDC: 629.78:[612.014.482+613.648

AUTOMATED SPECTROMETRIC UNIT FOR THE STUDY OF RADIATION CHARACTERISTICS OF COSMIC RADIATION ABOARD PROGNOZ-9 SPACE STATIONS

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 22, No 1, Jan–Feb 88 (manuscript received 4 Jan 87) pp 75–78

[Article by V. M. Petrov, Yu. I. Logachev, S. N. Karachevskiy, V. V. Bengin, I. K. Gvozdev, G. Ya. Kolesov, M. I. Kudryavtsev, A. I. Martynov, A. N. Podorolskiy, S. A. Sud and Ye. A. Devicheva]

[Text] At the present time there is no question that progress in investigation of the aggregate of processes in the active region of the sun, defined as a "solar proton even" [SPE] is possible only on the basis of combined analysis of its diverse components. Thus, hard x-radiation carries information about the characteristics of accelerated protons and electrons [5–9], centimeter- and meter-range radiowaves carry information about movement and release of accelerated particles in the photosphere and corona of the sun [8, 10], composition and angular-spectral characteristics of cosmic rays near earth's orbit furnish information about conditions of dissemination in interplanetary space and characteristics of particles accelerated in SPE [3, 4, 7]. Analysis is usually made to restore the measured spectra of solar cosmic rays (SCR) to the source and investigate the characteristics of the spectrum of accelerated particles thus determined with consideration of the various possible mechanisms of generation and injection [2, 6].

In order to improve the effectiveness of taking measurements on such a program it becomes necessary to form a special measuring complex that would permit not only obtaining data about the amplitude characteristics of various SPE factors, but time analysis of different factors and time relationship between them. Hence, it is necessary to measure SPE characteristics with high informativeness, reaching $20 \cdot 10^5$ bits per day. At the same time, during periods of absence of SPE such high informativeness is not needed, since processes in cosmic rays that have an appreciable influence on the radiation situation, in the absence of perturbations from solar flares, have periods that vary from a month to tens of years. Because of the requirement of efficient use of radiophysical equipment installed aboard long-lived space stations, it is necessary to develop complexes that can measure as many parameters as possible of the radiation fields in space with the limited resources allocated for such measurements.

Considering the foregoing approach, a spectrometric Sosna [pine] unit was developed, which is intended for investigation of SPE in the range of charged particle energy

from a few to hundreds of MeV/nucleon and hard x-radiation in experiments aboard Prognoz [prognosis] space stations (SS).

During an SPE, this unit was to permit measurements with high time resolution of the spectral characteristics of SCR protons in the enrgy range of 10–150 MeV and nuclei with energy of a few to tens of MeV/nucleon, respectively, dynamic characteristics of surges of hard x-radiation in the energy range of 10–200 KeV, angular distributions of SCR protons in the range of 10–150 MeV, charge spectra of particles accelerated in SPE in the z range of 1 to 26.

Metrological characteristics of Sosna complex

Instrument	Detector type	Energy range/ number of channels	Flux density	Geom.factor cm^2 "st"*	Energy resolution,%	Threshold change in mode
SKI-1	2 telescopes	10-150 MeV / 4	$1\text{-}10^5$ $cm^{-2}\cdot s^{-1}\times sr^{-1}$	0.26	27.2	2.4 cps for 10-30 MeV channel
RKh-1	2 phosph. detectors	10-200 KeV / 4	$1\text{-}10^3$ $cm^{-2}\cdot s^{-1}$	45	100-30	2 $cm^{-2}\cdot s^{-1}$ for 25-50 KeV

*Expansion unknown

In the absence of SCR, the complex was to operate on the measurement program, which involves examination of spectral characteristics of galactic cosmic rays (GCR) in the energy range of a few to tens of MeV/nucleon; investigation of charge spectrum of GCR in the range of z to 26; investigation of amplitude and space (from celestial sphere) characteristics of surges of x-radiation in the energy range of 10–200 KeV.

Of course, in the second mode, informativeness of the complex could be lower by a factor of 10^1–10^2 than in the first. The instrumentation of the Sosna complex and capabilities of Prognoz-9 SS solved this problem, and they also provided the most favorable conditions for implementing the planned measurement program. Prognoz-9 is quite convenient for the proposed studies: its markedly elongated orbit (apogee $\sim 7\cdot 10^5$ km) and inclination of $\sim 65°$ cause it to be outside the magnetosphere ~98% of a 24-h period, 99% of the time it is illuminated by the sun, stabilization of its twist on the sun [?] with a rotation period of 2 min makes it possible to study the spatial distribution of SCR on the basis of the method of recording particles using two telescopes [1]. Finally, the telemetry system (TM), which operates in two modes—with low (~0.1 Hz) and high (~0.8 Hz) frequency of interrogation—makes it possible to alter by almost a factor of 10 the volume of measured data, by changing the operating mode of the equipment, in accordance with radiation conditions in space. To execute the planned program, an SKI-1 proton and nucleus spectrometer, RKh-1 spectrometer for hard x-radiation and BU-1 unit to control measurement modes were included in the Sosna complex installed aboard Prognoz-9.

The table lists the main metrological characteristics of the Sosna complex.

The SKI-1 instrument has two detector telescopes to determine the angular distribution of recorded cosmic rays (CR). They are installed in such a manner that the axis of one of them forms an angle of 45° and the other, 225° with the axis of SS rotation. The electronic unit permits measurement of SCR in 4 ranges: 10–30, 30–60, 60–90 and 90–150 MeV. The RKh-1 x-ray spectrometer also has two detecting units placed symmetrically in relation to the axis of rotation. The detector axes form an

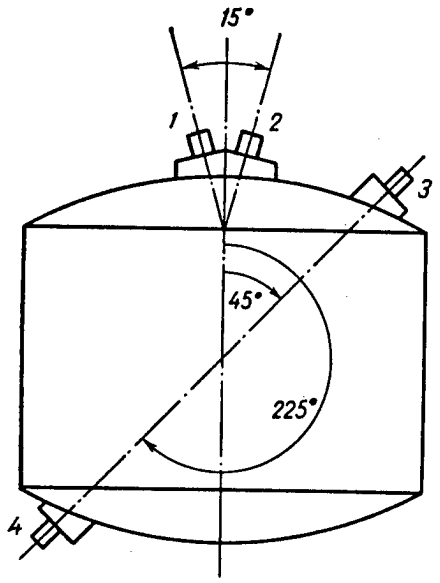

Figure 1.
Diagram of detector location in RKh-1 (1, 2) and SKI-1 (3, 4) instruments aboard Prognoz-9

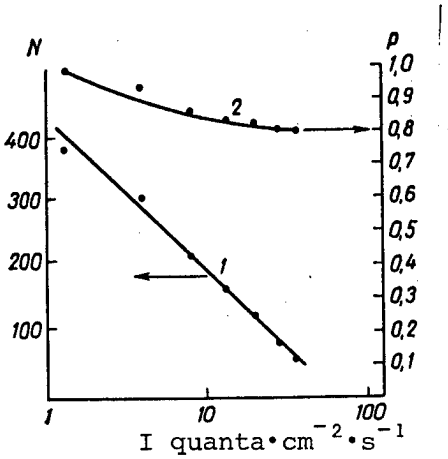

Figure 3.
Number of solar x-ray surges per year in flux exceeding specified (1) threshold and probability of exceeding it for solar surges accompanied by proton flux (2)

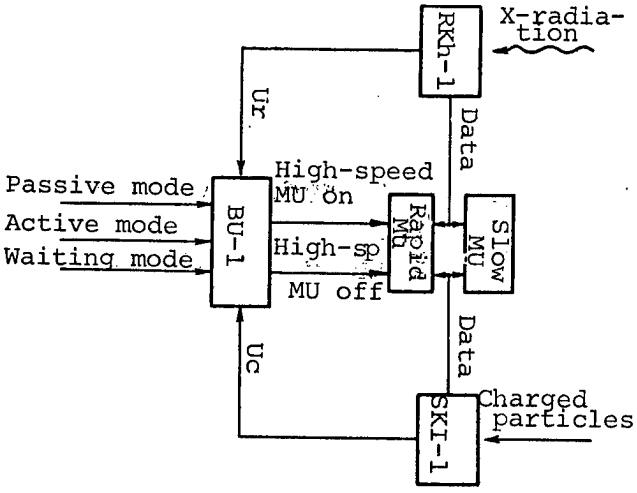

Figure 2.
Diagram of control of Sosna complex operation
MU) memory unit

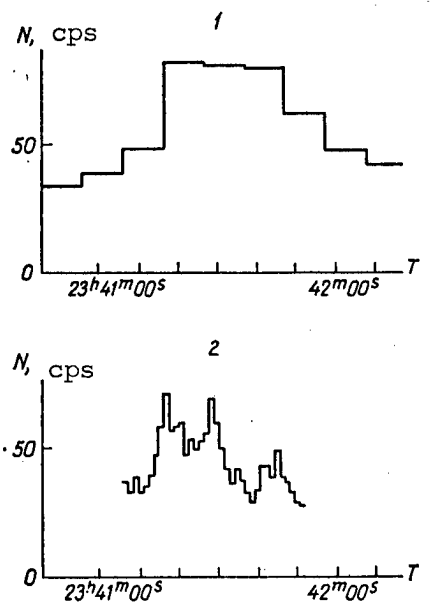

Figure 4.
Time profiles of x-ray surge (1.08.83) obtained using slow (1) and high-speed MU (2)

angle of 7.5° with it. Quantum flux is recorded in the following channels: 10–50, 25–50, 50–100 and 100–200 KeV. Such arrangement of detectors in relation to the axis of rotation of the SS makes it possible, using the method in [1], to calculate the

angular distribution of SCR proton flux and to determine the direction to the source of x-radiation if it is not on the sun. Figure 1 illustrates arrangement of detectors in the Sosna complex.

The choice of metrological specifications of the instruments is determined by the fact that processes measured with the Sosna complex differ appreciably in amplitude and intrinsic time. Thus, while GCR flux fluctuations with a period of 11 years, intrinsic times of GCR build-up number up to tens of hours, while the duration of x-ray surges is measured in the range of $10-10^3$ s. The values for CR flux are in the range of $1-10^5$ cm^{-2} s^{-1} sr^{-1}, while the flux of x-ray quanta is in the range of $10-10^4$ cm^{-2} s^{-1}. Since storage time of the onboard memory unit (MU) constitute 4 days (at 0.1 Hz interrogation frequency) to 12 h (at 0.8 Hz), it was also necessary to so organize measurement modes as to assure as complete as possible SPE recording when registering both x-ray quanta and protons. The measurement program for charge spectra allowed for analysis of a registered nucleus, one per interrogation cycle, with recording of ionization loss and total energy on the telemetry system. To record GCR nuclei, an interrogation frequency of 0.1 Hz is quite sufficient. These requirements for organizing recovery of data could be met if there were provisions for switching the MU from slow to rapid mode with change in flux of recorded radiations. In accordance with the purposes of the experiment and with considertion of these requirements, we set three modes for operation of the Sosna complex specified by the BU-1 control unit: 1) passive, when the instrument does not control complex operation; 2) active, when the high-speed TM system is turned on for 1 h to record an x-ray surge with greater than threshold amplitude, or for 10 h if the threshold for proton flux is exceeded; 3) waiting, when the high-speed TM system is switched on for 10 h of continuous operation when the measured parameters exceed any of the appropriate values.

The change from one mode to another is made upon command from the ground. The diagram of control of Sosna complex operation is illustrated in Figure 2.

As we mentioned above, as a rule there were communication sessions with Prognoz-9 once every 4 days. For this reason, with the chosen operating modes, the frequency of turning on the high-speed TM system should not be to high due to the need to limit the probabilty of missing detailed measurement of powerful SPE. For this purpose, the threshold for control of the high-speed TM system is set at a level that provides for it to be turned on no more, on the average, than once a day. Determination of this level of intensity of quanta was made in the following manner. According to [11], data were obtained for the period 1969–1972, which is similar in phase of solar activity cycle to the period of the Prognoz-9 mission, to the effect that frequency of x-ray surges is a function of maximum intensity, and that there is a probability of exceeding the specified intensity of quanta at peak surges associated with a proton flux (Figure 3, curves 1 and 2). We see from the data illustrated in Figure 3 that the required frequency (~once a day) of turning on the high-speed TM system is obtained when the maximum surge exceeds the baseline on the level of 2 $cm^{-2}s^{-1}$ in the 25–50 KeV channel. Virtually all surges associated with SCR will thus be recorded with high time resolution.

The threshold in the 10–30 MeV channel of the SKI-1 instrument was set at 24 $cm^{-2}s^{-1}sr^{-1}$ (approximately 10^2 higher than the background) to turn on the high-speed TM system according to results of measuring SCR, since this caused the high-speed TM system to operate only upon appearance of SCR (it can also be turned on by

protons from earth's internal radiation belt—ERB), and the statistical margin of error does not exceed 20%, which is sufficient to use the method in [1] for reconstruction of the angular distributions of protons.

The Sosna equipment was started up aboard Prognoz 9 on 1 July 1983, and it operated for 7 months. Since this period coincided with the trajectory of decline near the minimum of the 21st cycle of solar activity, major SPE were not observed. At the same time, we recorded about 400 surges of x-radiation of solar orgin, most of which had appreciable quantum fluxes in the energy range of 10–50 KeV. These surges triggered the high-speed TM system 40 times, so that data were obtained about quantum fluxes with resolution of 10 and 1.28 s. Figure 4 illustrates, as examples, two such surges recorded on 1 August 1983. The results of processing data pertaining to x-ray surges, increases in SCR and charge characteristics of CR during the period that Prognoz-9 was functional will be reported in future publications.

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LIQUID-PHASE OXIDATION OF ACETONE WITH HYDROGEN PEROXIDE ON OXIDE CATALYSTS

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 22, Jan–Feb 88 (manuscript received 24 Oct 86) pp 78–81

[Article by I. I. Vasilenko, N. M. Shevel and Yu. Ye. Sinyak]

[Text] Deep catalytic oxidation of organic substances is important to life-support systems [2, 14]. When regenerating water in water supply systems for spacecraft crews, it is desirable to effect oxidation of organic impurities in water at a low temperature before formation of end products of the CO_2, H_2O, etc., type [14]. These requirements limit considerably the range of catalysts and oxidants suitable for such purposes.

The oxidative catalytic method based on using molecular oxygen as oxidant involves expenditure of energy for evaporation of water and heating catalysts to temperatures of at least 150°C [14]. It is promising to use hydrogen peroxide [10, 16], which is a potent and ecologically pure oxidant [8, 13, 15] for destructive liquid-phase oxidation of organic impurities. Use of homogeneous oxidation catalysts is not recommended for water reclamation systems [6, 12, 19, 21], since this leads to secondary water pollution by heavy metal compounds. For this reason, it is more expedient to oxidize water impurities on heterogeneous catalysts for life-support systems of the closed type [5, 17].

Methods

In this work, 0.2 g catalyst was kept for 0.5 h in an aqueous solution of organic substance, then chemically pure hydrogen peroxide added and stirred with a magnetic mixer at 14–24°C. Total volue of the liquid phase was 20 ml; kinetics of emission of oxygen and carbon dioxide was monitored volumetrically; CO_2 was absorbed with 30% KOH.

Acetone was used as the model organic substance, since its concentration in the atmosphere of orbital space stations exceeded significantly the levels of other volatile impurities [2]. Acetone is removed from water upon oxidation of oxygen only in the vapor-gas phase on platinum catalysts at 250–300°C and at oxygen pressure of up to 20 atm [1, 11]. Several soluble peroxo derivatives are formed under the effect of hydrogen peroxide on aceton in the presence of homogeneous catalysts [18, 20], and the problem of purifying and regenerating water is more difficult.

The patterns of redox reactions during catalytic breakdown of hydrogen peroxide on lead oxides were the scientific basis for choosing active catalysits for destructive oxidation of acetone [4].

Results and Discussion

It was established that, in the presence of lead dioxide, acetone is not oxidized destructively, and carbon dioxide is not fixed in products of catalytic reactions, since hydrogen peroxide is used for reduction of Pb^{4+} [3]. When an oxide is used, with stoichiometric Pb_3O_4 is used, there is an insignificant yield of CO_2. With oxide of PbO in the yellow modification, substntial conversion of acetone to carbon dioxide is observed. Overall kinetic curves of O_2 and CO_2 emission in the system are illustrated in Figure 1.

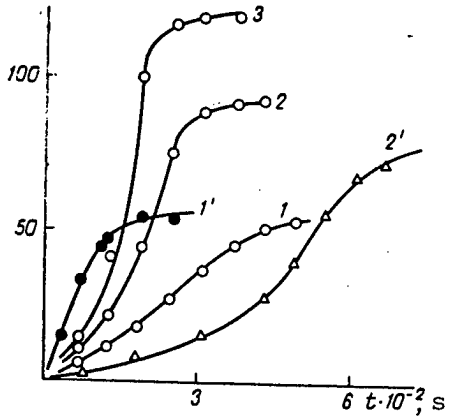


Figure 1.
Kinetics of gas emission in PbO—H_2O_2—CH_3COCH_3 system (50%)
1,2,3) 0.27, 0.52 and 0.61 M H_2O_2, respectively, at 22°C
1') 0.27 M H_2O_2 with 3-fold use of catalyst
2') 0.52 M H_2O_2 at 14.5°C

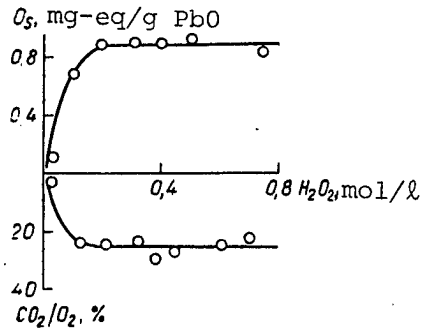


Figure 2.
Proportion of products of catalytic reactions, and amount of excessive oxygen on surface of lead oxide [11]

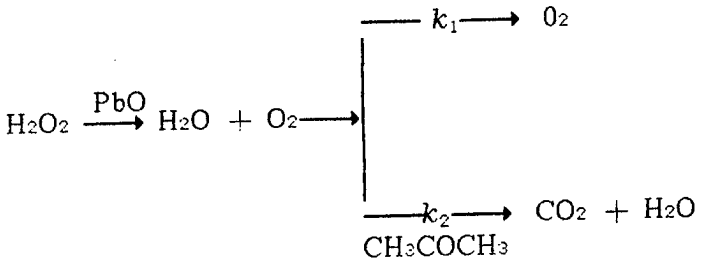
Figure 1 shows that catalytic processes in this system are characterized by an induction period, the duration of which is a function of concentration of hydrogen peroxide, temperature and frequency of contact of catalyst with the liquid phase. In particular, with repeated use of the same batch of lead oxide, no induction period is observed.

In order to check experimentally the theoretical conceptions of the principle of selecting oxide catalysts for oxidation of organic substances by hydrogen peroxide, we measured surface-active oxygen on PbO oxide by the pH-iodide method, the fundamentals of which are described in [22]. The amount of adsorbed oxygen was recorded after adding 0.5 g lead oxide to 10 ml hydrogen peroxide, using an aqeuous solution of potassium iodide in 0.1 M HCl.

It was established that the amount of adsorbed oxygen does not depend on amount of hydrogen peroxide in the tested range of its concentrations (Figure 2). With repeated use of the castalyst, no induction period is observed, while oxygen content, half-life

of hydrogen peroxide and CO_2/O_2 ratio remain constant. Consequently, with oxidation of acetone an induction period is necessary for accumulation of active oxygen on the surface of the catalyst.

With consideration of the foregoing, heterogeneous catalytic reactions in the system in question can be described by the following diagram:



where k_1 is the constant of rate of recombination of active oxygen and k_2 is the constant of rate of catalytic oxidation of acetone.

The correlation between volumes of gaseous reaction products does not depend on concentration of hydrogen peroxide (see Figure 2); consequently, k_1/k_2 is determined only by catalyst properties and experimental conditions (mixing mode, temperature, etc.).

From the equations for oxidation, $8H_2O_2+CH_3COCH_3=3CO_2+11H_2O$, and dissociation, $2H_2O_2=2H_2+0_2$, it follows that with the observed ratio of $CO_2/0_2=25\%$, the factor of induction of conjugate catalytic processes of breaking down hydrogen peroxide and oxidizing acetone is 0.33. This means that, at 21–23°C and mixing rate of 120 rpm, only 23–25% hydrogen peroxide is used to oxidize acetone, and about 75–77% of the total amount is required for its catalytic dissociation and release of oxygen.

The extent of acetone conversion to carbon dioxide depends on molar ratio between system components (see Table). The listed data also indicate that, in order to obtain a high yield of CO_2 under experimental conditions, a significant surplus of hydrogen peroxide is needed. For better use of oxidant, it is expedient to effect numerous successive dilutions of hydrogen peroxide. A high degree of conversion is obtained, even with relatively high baseline acetone content (Figure 3). The extent of acetone conversion in the case of one contact with reagents also depends on amounts of acetone, hydrogen peroxide, and stirring mode, and it constitutes from 14 to 92% at a temperature of 20–24°C in 10 min of oxidation (Figure 4).

In order to compare the proposed method of oxidizing acetone to those already known, which are based on homogeneous oxidation of organic subtances with hydrogen peroxide in the presence of heavy metal salts [9], we added to water with acetone hydrogen peroxide and copper sulfate. The molar ratio between components corresponded to 23.2 mg/l acetone, 0.420 M H_2O_2 and 0.03 M copper sulfate. In this system, totaling a volume of 20 ml, catalytic reactions and emission of gas end in 9 h. Only oxygen is released during oxidation; solution pH drops from 4.12 to 3.32, while residual acetone constitutes 12.4%. These data indicate that, in this system, acretone is oxidized with formation of organic acids as stable end products; the oxidation process is slow and does not lead to deep purification of water.

Effect of ratio between components on extent of acetone conversion

Initial quantit., mg		Molar ratio between components	Residual CH_3COCH_3, mg	Conversion, %
CH_3COCH_3	H_2O_2	CH_3COCH_3 — H_2O_2 — PbO		
0,059	281	0,112:827:90	0,005	92
0,085	281	0,146:827:90	0,018	79
0,112	294	0,193:865:90	0,039	67
0,156	294	0,269:865:90	0,077	50
0,348	294	0,600:865:90	0,180	48
0,503	288	0,868:845:90	0,290	42
0,928	281	1,600:827:90	0,576	39

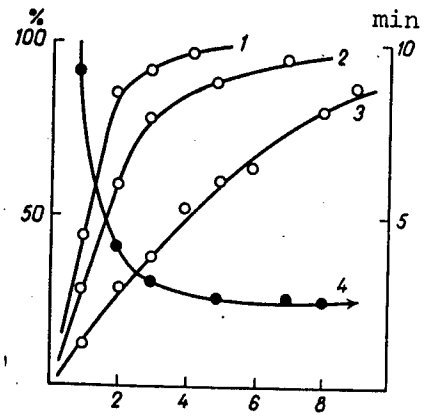


Figure 3.
Extent of conversion of acetone to CO_2 (left, %) and oxidation time (right, min) with repeated addition of hydrogen peroxide
1,2) initial acetone content: 27.5 and 49.5 mg/*l*, respectively
3,4) 60.2 mg/*l* with 0.5 M H_2O_2

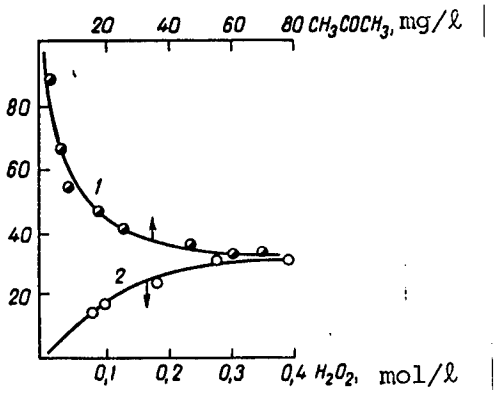


Figure 4.
Extent of conversion (%) as a function of initial concentration of acetone (mg/*l*) and hydrogen peroxide (mol/*l*)
1) 0.432 M H_2O_2
2) 60.2 mg/*l* CH_3COCH_3

Thus, the method we propose for oxidation of acetone reduces treatment time considerably, it enhances the intensty and depth of oxidation of acetone, extent and quality of water treatment.

In conclusion, it should be noted that the minimal residual concentration of acetone after oxidation with hydrogen peroxide in the presence of PbO even with single contact with reagents is 0.24 mg/*l*. This is considerably lower than the maximum permissible concentration of acetone in public water supply reservoirs, and it is close to that of many other organic substances a general sanitary and sanitary-toxicological limiting parameter of harmfulness [7]. For this reason, the above findings are of applied interest for local removal of organic compounds from water in the case a closed water-supply cycle.

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BRIEF REPORTS

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HUMAN ERYTHROCYTE METABOLISM IN THE PRESENCE OF HYPEROXYGENATION DURING ANTIORTHOSTATIC HYPOKINESIA

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 22, Jan–Feb 88 (manuscript received 20 Dec 86) pp 81–82

[Article by V. Ye. Vorobyev, V. F. Ivchenko and L. L. Stazhadze]

[Text] A study of the effects of high concentrations of oxygen at normal barometric pressure should include evaluation of respiratory function of blood, which serves as the central element in the transport of gases, connecting external and tissue respiration [1]. The question of effect of high oxygen concentrations on respiratory function of blood is of some interest to space biology and medicine. In particular, it is important to understand how red cell metabolism, which is one of the limiting factors of maximum permissible exposure of man to a hyperoxic environment, changes under normobaric hyperoxic conditions. However, there is very sparse information about erythrocyte metabolism in weightlessness or conditions that simulate it. Our objective here was to investigate some of the mechanisms in the system of the blood's response to hyperoxia in healthy people in a series of studies using antiorthostatic [head-down tilt] hypokinesia (HDT).

Methods

A state of normobaric hyperoxia was produced in subjects, by means of artificial pulmonary ventilation with oxygen for 45 min using an RO-5 unit, in two series of tests with 14-day HDT. Artificial ventilation was administered in normoventilation mode under general anesthesia using such agents as sodium hydroxybutyrate and seduxen, in the presence of total muscle relaxation. The studies were performed twice: before the start of HDT and on the 12th day of hypokinesia. Assay of 2,3-diphosphoglyceric acid (2,3-DPG) was made by the method of N. P. Meshkova and N. V. Aleksakhina [2] in red cells isolated from venous blood. The condition of adenylic system fractions—adenosine triphosphate (ATP) and adenosine diphosphate (ADP)—was evaluated by high-voltage electrophoresis [3]. Concentration of inorganic phosphate (IP) in venous plasma was determined by the method of Fiske and Subbarow [4]. Glucose, lactate and pyruvate were measured by the enzyme-spectrophotometric method using standard sets of reagents. A total of 16 essentially healthy men participated in the studies. During tests in the baseline period, the subjects were in horizontal position, and on the 12th day of hypokinesia in antiorthostatic position (−8°). Artificial ventilation was continued for 30 min after stopping delivery of oxygen, with inhalation of ordinary air. Reliability of demonstrated changes was assessed using Student's criterion.

Dynamics of changes in tested parameters of erythrocyte and tissue metabolism under the effect of oxygen ($M \pm m$)

Parameter	Stage of study					
	before HDT	45th min of oxyg. exposure	30th min after oxygen	12th day of HDT	45th min of oxygen exposure	30th min after oxygen
Glucose, mmol/ℓ	4,15±0,37 (8)	4,56±0,37 (8)	4,64±0,49 (8)	3,10±0,40 (8)	3,68±0,41 (8)	3,67±0,73 (8)
Lactate, mmol/ℓ	1,83±0,20 (10)	0,97±0,13* (10)	0,97±0,15* (8)	1,50±0,20 (10)	1,18±0,14 (10)	1,10±0,10 (8)
Pyruvate, mmol/ℓ	0,04±0,01 (6)	0,07±0,01* (8)	0,07±0,01* (8)	0,04±0,01 (8)	0,03±0,003** (8)	0,02±0,001** (8)
IP, μmol/mℓ	4,44±0,11 (12)	4,03±0,40 (12)	4,24±0,31 (8)	4,47±0,12 (12)	4,65±0,12 (14)	4,57±0,24 (8)
2,3-DPG, μmol/mℓ	3,26±0,07 (12)	2,70±0,10** (12)	2,86±0,11* (8)	3,33±0,10 (12)	4,38±0,25** (14)	4,27±0,28* (8)
ATP, μmol/mℓ	0,93±0,12 (12)	1,22±0,17 (12)	1,03±0,21 (8)	1,00±0,10 (12)	0,94±0,10 (14)	0,82±0,09 (8)
ADP, μmol/mℓ	0,32±0,12 (12)	0,13±0,05 (12)	0,23±0,10 (8)	0,26±0,08 (12)	0,44±0,10 (14)	0,45±0,20 (8)
ATP/DPG	0,29±0,04 (12)	0,45±0,06 (12)	0,37±0,08 (8)	0,30±0,03 (12)	0,23±0,03 (14)	0,20±0,02* (8)

Note: Number of cases given in parentheses. $*p<0.05$, $**p<0.01$

Results and Discussion

Blood glucose level consistently rose during hyperoxia both before hypokinesia and during HDT (see Table).

It was established that during HDT there was increase in concentration of 2,3-DPG ($p<0.05$) and ADP in erythrocytes, as well as decrease in ATP, under the effect of hyperoxia. Concurrently, we found an increase in blood glucose content and decline in levels of lactate and pyruvate, as compared to the baseline prior to use of hyperoxia. The results are indicative of a possible shift in glycolysis of erythrocytes of enzymatic equilibrium, which is maintained by phosphoenol pyruvate in the direction of formation of glyceric acids. We cannot rule out the possibility of development of pyruvate kinase insufficiency under such conditions.

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EFFECT OF DIFFERENT MODES OF VOLUNTARY CONTROL OF BREATHING ON HUMAN ELECTROENCEPHALOGRAM WITH EXPOSURE TO ACUTE HYPOXIC HYPOXIA

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 22, Jan–Feb 88 (manuscript received 27 Oct 86) pp 82–84

[Article by Ye. P. Gora]

[Text] The search for means of enhancing effectiveness of human adaptation to low barometric pressure is of great scientific and practical importance to space biology and aerospace medicine. It is assumed that voluntary control of breathing may be one of the means of achieving this [1, 4].

At present we have no clearcut idea about the distinctions of the effect of voluntary control of breathing on change in functional state of the CNS [central nervous system] and, in particular, electrical activity of the brain during adaptation to acute hypoxic hypoxia.

Our objective here was to investigate the mechanisms of this feedback using some modes of voluntary control of respiration during exposure to acute hypoxia corresponding to an "altitude" of 5000 m.

Methods

A total of 22 essentially healthy men 18—20 participated in the study. After a baseline examination, which included recording of physological functions at rest, the subjects breathed a gas mixture containing 10.5% 0_2 during breath-holding in inspiration and voluntary 2-min ungraded hyperventilation. Starting with the 10th min of hypoxia, which lasted 30 min, we performed breathing tests on the subjects: voluntary breath-holding and, after restoration of breathing, voluntary 2-min hyperventilation.

During the study, we recorded the pneumogram, electrocardiogram (ECG) in the second standard lead and electroencephalogram (EEG) in two bipolar fronto-occipital leads in the right and left hemispheres.

Results and Discussion

During 2-min ungraded hyperventilation under normal conditions, we recorded the EEG changes inherent in this test—disorganization of L rhythm and appearance of slow waves. These changes depended on the individual.

EEG changes in 22 subjects during 2-min voluntary hyperventilation under normal conditions and when breathing a gas mixture containing 10.5% O_2

Normal conditions		Gas mixture	
nature of EEG changes	number of subjects	nature of EEG changes	number of subjects
Slow waves	15	Slow waves	4
L-rhythm disorganization	5	L-rhythm disorganization	7
L-rhythm activation → L-rhythm disorganization	2	L-rhythm activation → L-rhythm disorganization	2
		L-rhythm activation	5
		No changes	4

When breathing a gas mixture containing 10.5% O_2 during hyperventilation, the EEG changes were less marked. The table lists data on EEG changes aduring 2-min voluntary hyperventilation of 22 subjects under normal conditions and at the start of hypoxia when breathing a mixture containing 10.5% O_2.

In both instances, we observed a tendency toward increase in heart rate during the test, as compared to the baseline, by 29.9 and 36.7%, respectively.

Voluntary breath-holding under normal conditions did not lead to significant changes on the EEG. Activation of L rhythm occurred in only 3 out of the 22 subjects.

During exposure to hypoxia, duration of voluntary breath-holding was reduced on the average from 38 ± 2.6 to 27 ± 2.3 s ($p < 0.01$). The changes that occurred on the EEG under the effect of hypoxia (desynchronization, disorganization of L rhythm, slowing of activity) became more marked during the test in 4 out of 22 subjects.

The changes in heart rate were in different directions during breath-holding under normal and hypoxic conditions.

In selecting hyperventilation and breath-holding as modes of voluntary control of respiration, we were governed by the fact that these tests lead to opposite changes: hyperventilation increases oxygenation and carbon dioxide output, while breath-holding causes decrease in oxygentation, retention of carbon dioxide and prevents development of hypocapnia, which is associated with involuntary hyperventilation in the presence of altitude hypoxia.

The EEG changes that appear under normal conditions during hyperventilation are attributed to the effect of hypocapnemia on reticular structures [2]. The opinion has been voiced that cerebral hypoxia, which occurs during hyperventilation due to increase in tonus of cerebral vessels, is also an important factor in changing bioelectric activity of the brain [2–3, 6].

There are some differences in nature of hyperventilation under normal conditions and in the presence of hypoxic hypoxia. It was shown that voluntary hyperventilation is 5–10% greater under hypoxic conditions than under normal ones; this leads to more significant decline of p_ACO_2 than under normal conditions [1, 4]. Yet, as can be seen in the table, the EEG changes associated with voluntary hyperventilation under hypoxic conditions were less marked. An analagous effect was obtained by Holmberg in a test

involving 3-min voluntary hyperventilation using a breathing mixture that contained 7.5% O_2 [5].

Evidently, the fact that there is activation of the reticular formation of the brain stem and suprapontine structures under the effect of the adrenosympathetic system at the start of adaptation to acute hypoxia plays some part in this reaction of bioelectrical activity of the brain [7].

In addition, acute hypoxia most probably prevents, to some extent, increase in tonus of cerebral vessels, which occurs under the effect of hypocapnia.

In the case of acute hypoxia, breath-holding time is reduced under the effect of the hypoxic factor. At the same time, it leads to intensification of the effect of hypoxia, as can be seen from the more intensive changes on the EEG of a number of subjects.

Thus, the modes of voluntary control of breathing we have discussed (hyperventilation, breath-holding) during exposure to acute hypoxic hypoxia lead to specific sets of functional changes in physiological systems (respiratory system, cardiovascular system, CNS) and, consequently, in bioelectrical activity of the brain. These changes are attributable to both humoral (hypocapnia, intensification of hypoxia, etc.) and neurogenic factors.

The extent of manifestation of such changes in both cases is determined on an individual basis in different people, and apparently it is indicative of individual differences in adaptation mechanisms. This must be borne in mind when selecting a specific mode of voluntary control of breathing in order to enhance effectiveness of adaptation to altitude hypoxia.

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EXPERIMENTAL STUDY OF PROTECTIVE EFFECT OF ANTIOXIDANT ENZYMES—SUPEROXIDE DISMUTASE AND CATALASE—WHEN USING INTERMITTENT TOXIC MODES OF HYPERBARIC OXYGENATION

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 22, Jan–Feb 88 (manuscript received 29 Apr 86) pp 84–86

[Article by F. A. Zvershkhanovskiy, M. A. Simonyan and Yu. A. Pilipenko]

[Text] Formation in the body of active forms of oxygen (superoxide anion radical, hydrogen peroxide, hydroxyl radical) is the triggering factor of oxygen intoxication when using toxic modes of hyperbaric oxygenation (HBO) [4]; they have the capacity to react with endogenous substrates (primarily phospholipid biomembranes) with formation of organic peroxides [5]. Peroxide compounds have an inactivating effect on oxide reductase, as a result of which the cell loses the capacity to utilize surplus oxygen [12]. Superoxide dismutase (SOD), catalase and glutathione peroxidase play an important role in dismutation of superoxide radicals [11, 7]. These enzymatic antioxidants (AO) manifest their stabilizing effect by inhibiting free-radical oxidation of lipids (FROL) in biological membranes. Exposure to toxic HBO is associated with decrease in activity of these AO, which leads to accumulation of lipid peroxides in excess of the physiological reserve of the antioxidant system (AOS) [3, 6]. In this study, we have tried to prevent the toxic effect of hyperbaric oxygen by means of administration of exogenous SOD and catalase.

Methods

This investigation was conducted on 107 male white rats weighing 160–200 g. The animals were divided into six groups. The 1st group consisted of 15 intact rats. The 2d–6th groups of animals were submitted to toxic doses of hyperbaric oxygen (daily exposure to 0.45 MPa HBO for 2 h/day for 6 days, in the mornings). The 2d group of rats (12 animals) was exposed only to HBO; the 3d-6th groups were given the tested agents just prior to HBO: 3d group (12 animals)–SOD in a dosage of 0.5 mg/kg weight, intragastrically; 4th (12 rats)—catalase in a dosage of 0.5 mgg/kg intragastrically through a tube; 5th (12 rats)—SOD and catalase via the same route in a dosage of 0.5 mg/kg of each agent; 6th (10 animals)—SOD intraperitoneally in a dosage of 0.5 mg/kg. Beer's convulsive effect and the general toxic effect of oxygen intoxication were used as criteria of the toxic effect of hyperbaric oxygen. We assessed the exposure session during which seizures occurred in the altitude chamber, or death in the chamber, or death within 24 h after HBO. Concurrently, we ran a second series of experiments on identical groups of animals which were decapitated under intraperitoneal barbamyl anesthesia (100 mg//kg) immediately after second exposure to

HBO. This period was selected because antioxidant activity, which is activated by HBO, diminishes by the end of the 1st day, as well as with repeated sessions of HBO [3].

The glandular part of the stomach was homogenized under refrigeration, and the following parameters of FROL and AOS were determined: malonic dialdehyde (MDA) [9], diene conjugation of higher fatty acids (DA) [8], Schiff bases [14], reduced (GSH) and oxidized (GSSG) glutathione [13]. The results were processed using tables in [2] and methods of variational statistics [10].

Table 1. Effect of SOD and catalase on rats with intermittent toxic modes of HBO ($\bar{x} \pm m$)

Animal group	First HBO session				Second session				Third session			
	A	B	C	D	A	B	C	D	A	B	C	D
2	12	$0^{3,4,5}$	0^{5}	0^{4}	$12^{4,5}$	8	0	2	$10^{3,4,5}$	10^{6}	2	5
3	12	$8^{2,6}$	2	0^{4}	10	8	2	4	$4^{2,6}$	4^{6}	2	2
4	12	$8^{2,6}$	1	$6^{2,3,6}$	5^{2}	5	0	5^{2}	$0^{2,6}$			
5	12	$10^{2,6}$	$6^{2,6}$	0	6^{2}	6	2	$2^{2,6}$	$2^{2,6}$	2^{6}	2^{6}	0
6	10	$0^{3,4,5}$	0	0^{4}	10	$0^{2,3,4,5}$	0	0^{4}	$10^{3,4,5}$	$0^{2,3,5}$	0^{5}	5

Animal group	Fourth session				Fifth session				Sixth session			
	A	B	C	D	A	B	C	D	A	B	C	D
2	3	3^{6}	0	3^{6}	0^{6}				0			
3	0^{6}				0^{6}				0			
4	0^{6}				0^{6}				0			
5	0^{6}				0^{6}				0			
6	$5^{3,5,4}$	0^{2}	0	0^{2}	$5^{2,3,4,5}$	0	0	3	2	0	0	2

Key: A) number of animals at start of session
B) number of animals with seizures in altitude chamber
C) number animals that died in altitude chamber, deaths within 24 h after being in pressure chamber [Translator's note: latter probably refers to column D, although no reference to D is made in source key]

Note: Here and in Table 2: superscripts refer to group number in comparison to which a reliable difference ($p < 0.05$) was found

Results and Discussion

As shown by our findings (Table 1), intragastric administratoin of SOD, catalase and a combination of the two led to appearance of seizures in rats, already during the first session of HBO; there was an increase in incidence of deaths, both in the altitude chamber (5th group) and within 24 h after the session (4th group). The survival rate was reliably lower in the 4th and 5th groups by the 2d and 3d sessions of HBO, as well as in the 3d group by the time of the 3d session, as compared to the 2d and 6th groups. No deaths or seizures in the altitude chamber were observed in the course of

6 HBO sessions when SOD was given intraperitoneally; there was reliable increase in survival rate, as compared to animals of the control group.

Table 2. FROL and AOS parameters in rat stomach tissues ($\bar{x} \pm m$)

Animal group	FROL			AOS, nmol/g	
	MDA, mmol/g	DA, mmol/g	Schiff bases μmol NADH/g	GSH	GSSG
1	0,040±0,003	0,267±0,014	0,170±0,007	19,52±1,62	3,66±0,36
2	0,077±0,005[1, 4]	0,310±0,009[1, 4, 5]	0,232±0,014[1, 3, 4, 5, 6]	10,05±0,84[1, 6]	4,15±19[4, 5]
3	0,078±0,006[1, 4]	0,322±0,009[1, 4]	0,283±0,012[1, 2, 4, 6]	9,76±0,95[1, 6]	4,31±0,15[4, 5]
4	0,097±0,004[1, 2, 3, 5, 6]	0,384±0,012[1, 2, 3, 6]	0,345±0,020[1, 2, 3, 6]	9,08±0,74[1, 6]	6,17±0,34[1, 2, 3, 6]
5	0,075±0,006[1, 4]	0,354±0,014[1, 2, 6]	0,327±0,019[1, 2, 6]	9,06±0,74[1, 6]	6,17±0,34[1, 2, 3, 6]
6	0,067±0,003[1, 4]	0,297±0,019[4, 5]	0,196±0,009[2, 3, 4, 5]	14,47±0,95[1, 2, 3, 4, 5]	3,95±0,18[4, 5]

Determination of FROL and AOS (Table 2) revealed activation of free-radical processes in the presence of decrease in GSH content of gatric tissues in rats submitted to toxic doses of oxygen (2d group). Intragastric administration of SOD, catalase, as well as a combination of the two, did not prevent initiation of FROL; we observed an increase in GSSG and decrease in concentration of GSH, which is an important indicator of oxygen intoxication [6, 7]. Inytraperitoneal administration of SOD improved a number of FROL and AOS parameters, including increase in GSH content.

Thus, the attempt to prevent the toxic effect of HBO by administering SOD, catalase or a combination of the two intragastrically did not lead to the desired results, probably due to the rapid breakdown of enzymes in an acid medium and decline in their activity [15]. Partial hydrolysis of the AO used, presence in them of metal ions with variable valence reduced by superoxide radicals are instrumental in the chain and branched nature of FROL processes. In addition, generation of chains of oxidation also occurs when excessive hydroperoxides are broken down [1].

When given intraperitoneally, SOD causes dismutation of superoxide anions, manifesting its protective action with respect to endogenous enzmatic systems, including SOD, catalase, glutatione perioxidase, and it effects adaptative homeostasis under hyperoxic conditions.

These findings indicate that only intraperitoneal administration of SOD limits free-radical processes, provides for better survival of animals exposed to intermittent toxic doses of oxygen.

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METHOD FOR MEASURING ABSOLUTE LINEAR PARAMETERS OF CHROMOSOMES

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 22, Jan–Feb 88 (manuscript received 22 May 86) pp 86–87

[Article by L. I. Chabala]

[Text] Some attention is given to change in chromosome morphology in studies of the effects of spaceflight factors on the body [1]. In this regard, consideration of their absolute linear parmeters would enable us to gain additional information.

At the present time, the method of measuring chromosomes in relative units on microphotographs or drawings from a negative is used extensively [2]. While the method is readily available, its flaw is that the true size of the object remains undetermined.

We know of a method of measuring microscopic objects in microns under a microscope, using the scale of an eyepiece micrometer. When examining chromosomes under an MBB-1 microscope with a 90*x* lens, a 7*x* eyepiece is used, which has an attachment for the ocular micrometer. In this case, the ocular micrometer has a scale factor of 2 μm. But in some animal species (for example, albino rats), the chromosomes are about 2 μm in size in most cases. For them the above scale factor is rather large and does not permit accurate measurements. Moreover, this method requires enormous eye strain, and when there are many chromosomes in the karyotype that are morphologically similar, it is extremely difficult to systematically isolate and measure each of them.

For this reason, a method was developed for measuring the absolute linear parameters of chromosomes, which will permit mass scale analysis with higher precision.

Methods

The proposed method permits measurement of linear parameters of chromosomes in micrometers on photos or drawings. We took microphotos of the ruler of the eyepiece micrometer and chromosome sets at the same magnification (lens 90*x*, camera 1.30, eyepiece 15*x*). Then, they were enlarged from the negative also under the same conditions, and chromosomes were measured on photos or drawings using a graduated ruler designed on the basis of the ruler of the ocular micrometer.

The scale factor on the eyepiece micrometer ruler is 0.01 mm, or 10 μm. Photos of such 10-μm segments after magnification are illustrated in Figure 1. On one of them, we constructed a scale-ruler from the theorem of Thales, to measure the chromosomes. For this, we drew ray AO at an angle from the extreme point A and plotted on

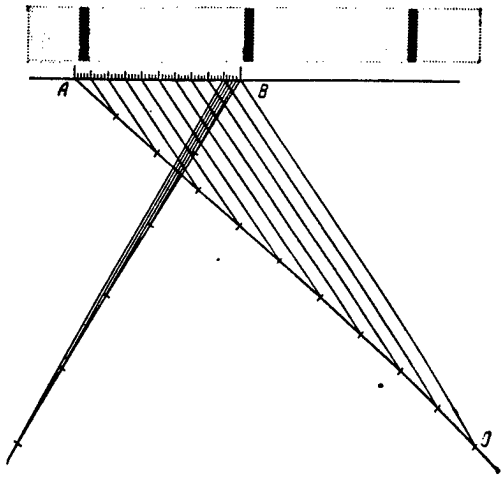

Figure 1.
Microphoto of eyepiece micrometer ruler and scale-ruler for measurement of microscopic objects (lens 90*x*, camera 1.30, eyepiece 15*x*)

it 10 coherent segments, and the last point, O, was connected with point B of calibrated segment. Then, we drew lines parallel to OB from the other 9 points of ray that separated the calibrated section into 10 congruent segments each of which corresponded to 1 μm. The latter were divided similarly into 5 more parts, and we obtained 0.2-μm segments. This scale-ruler was used to measure the linear parameters of chromosomes.

Figure 2 is a photograph of a chromosome set at the same magnification. The chromosome arms, with consideration of centromere and chromosome length, were measured in order of the indicated numbers, and the true size of the chromosomes was determined using the scale-ruler.

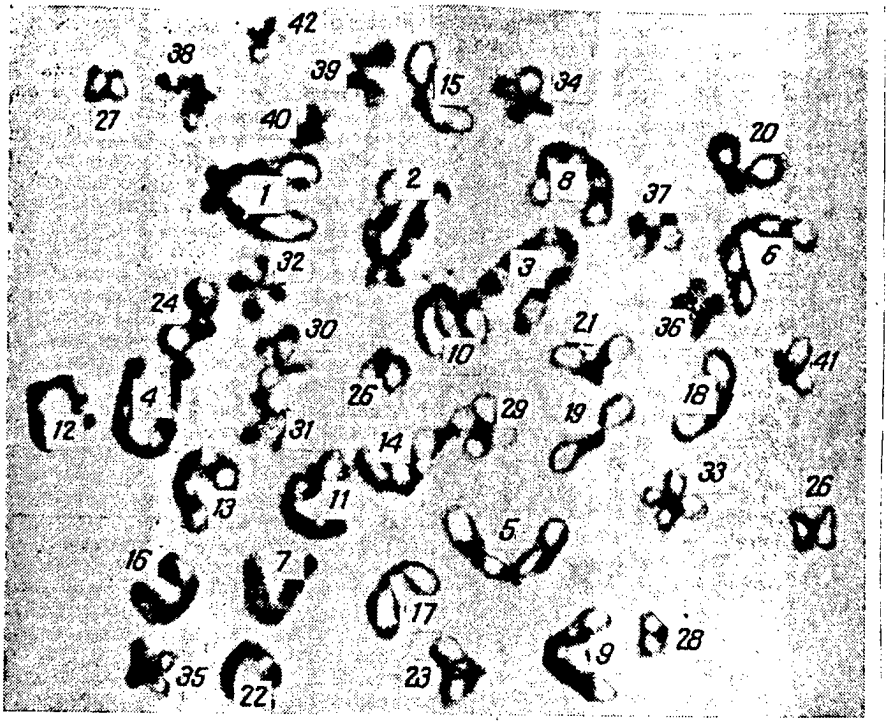

Figure 2. Microphotograph of chromosome set of mongrel white rats (lens 90*x*, camera 1.30, eyepiece 15*x*)

Chromosomes were measured with a margin of error of tenths of a micrometer, and most of them (Nos 13-42, see Figure 2) had linear parameters ranging from 2 to 1 μm.

Use of a ruler-scale with a scale factor of 0.2 μm makes it possible to take the linear measurements of all chromosomes in a set, which permits better identification.

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CURRENT EVENTS AND INFORMATION

UDC: 613.693:92 Dobrotvorskiy

CONTRIBUTION OF N. M. DOBROTVORSKIY TO DEVELOPMENT OF SOVIET AVIATION MEDICINE

Moscow KOSMICHESKAYA BIOLOGIYA I AVIAKOSMICHESKAYA MEDITSINA in Russian Vol 22, Jan–Feb 88 (manuscript received 16 Feb 87) pp 87–90

[Article by V. A. Bodrov]

[Text] The advances in development of aviation and cosmonautics are inseparably linked with the achievements of Soviet aviation medicine, which not only brought to life the principles of Soviet preventive medicine, but had an appreciable influence on progress of aerospace engineering. The role and place of Soviet aviation medicine as a scientific discipline sector of clinical work of aviation physicians are particularly noticeable and significant when we make an historical analysis of its development.

Among the many urgent and vital tasks put to the young Soviet state after the Great October Socialist Revolution, development of its own Soviet aviation was one of the first. Aware of the importance of aviation and need for its development, the Soviet government and V. I. Lenin personally devoted, from the very first steps, much attention to preservation of old and rearing young aviation cadres, and organization of research laboratories. Already in the fall of 1918, the position of air squadron physician was introduced, medical supervision of flight personnel was organized, and in 1920 a special sanatorium was created for pilots.

This was the start of systematic medical investigation of flight personnel. This is how Soviet aviation medicine was born. Its inception started virtually from scratch. And for this reason, the contribution to development of Soviet aviation medicine, the first Soviet flight surgeon, S. Ye. Mints, Professor A. P. Nechayev, Yu. A. Vasilyev and others was all the more noticeable and important.

But we would say that N. M. Dobrotvorskiy held the most prominent and important place among the founders of Soviet aviation medicine. His life was short but fruitful and left its bright mark in aviation medicine, having laid its foundation and defined the routes of development of a number of scientific problems and practical directions.

N. M. Dobrotvorskiy was born in 1893. He started his career after graduating from the RKKA [Workers' and Peasants' Red Army] Military Medical Academy in October 1917 in the post of senior medical officer of the 1st Ukrainian Grenadiers' regiment and continued in 1918 as instructor of clinical psychiatry at the above academy.

The early years of the Soviet state were years of inception of Soviet aviation medicine, the basic concept of which was the need for a deeper approach to optimization of pilot work, systematic investigation of questions of accidents and traumatism, more comprehensive monitoring of physical condition of pilots. Establishment in 1924 of four Air Force psychophysological laboratories—the first such laboratories in the RKKA—at all flying schools, as well as of the Central Psychophysiological Laboratory, was instrumental in implementation and introduction to practice of the above ideas. N. M. Dobrotvorskiy was directly involved in organizing the laboratories, and he became the head of the Central Laboratory in July 1924.

Establishment of these laboratories immediately delineated the basic direction of Soviet aviation medicine, which was named "psychophysiological." N. M. Dobrotvorskiy defined the tasks for the psychophysological laboratories: "... investigation of physical and psychophysiological condition of flight personnel, screening on the basis of physical and psychophysiological data received by the Air Force, investigation of working conditions in the air service, detection of deleterious factors in this service and their elimination."[1]

He wrote that organization of these laboratories was a major event and a definite stage of implementation of the principle of preventive, prophylactic medicine.

N. M. Dobrotvorskiy took on a very complicated and responsible task from the very start of his career, that of creating Soviet medicine on the basis of the teaching of I. P. Pavlov on higher nervous activity. In those days, when Pavlovian physiology did not yet have proponents among wide circles of medical workers, such orientation of aviation medicine definitely had revolutionary elements.

From the very first steps in his sceintific and clinical endeavors, N. M. Dobrotvorskiy decided to separate himself from the psycho-engineering trends that prevailed in miliatry medicine at that time. "The flaw in these methods," N. M. Dobrotvorskiy would say, "is that there is an excess of subjectivism and all sorts of psychological and reflexological elements." The only permissible method in the laboratory, he continued, "should be the objective method of I. P. Pavlov." He also maintained that "all the procedures and skills that an individual must learn to become a pilot are nothing other than conditioned reflexes." Let us not judge N. M. Dobrotvorskiy too harshly for such categorical and unilateral thinking, for his times it was rather daring, progressive and, to this day, this view explains many phenomena in man's substantive activities.

N. M. Dobrotvorskiy validated several concepts of this scientific direction in defining the tasks of flight personnel psychophysiology. In particular, he believed the term, psychophysiology, should be used in the broad sense, i.e., as physical, physiological and psychological examination of an individual, although it should be construed more correctly, scientifically, as physiological investigation of the mind. Today the concept of "psychophysiology" is more often given a broad, collective meaning, although the definition of this concept by a number of our prominent Soviet physiologists and psychophysiologists (N. P. Bekhterev, Ye. N. Sokolov) is narrower and deeper.

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[1]From the article by N. M. Dobrotvorskiy, "Tasks for the Air Force Psychophysiological Laboratories," in the journal, VESTNIK VOZDUSHNOGO FLOTA, 1925, No 10, pp 11–14.

With reference to mental capacities, N. M. Dobrotvorskiy observed that they should not be construed as something apart, unrelated to physical and physiological phenomena in the body. Unfortunatley, even at the present time, human capacities are usually viewed only from the standpoint of purely mental phenomena of the personality and as being unrelated to the structural and functional distinctions of the body.

N. M. Dobrotvorskiy was one of the founders of a number of scientific directions in aviation, which have presently gained universal recognition, such as vocatonal psychological screening of pilots, aviation engineering psychology, habitability of flight vehicles, aviation ergonomics.

For the first 3 years at the Central Laboratory, he worked exclusively on psychophysiological problems and dealt with analysis of psychophysiological analysis of the distinctions of flying work, methods of flight training, screening of fighter pilots and aircraft observers, etc. As he delved deeper into questions of aviation, he expanded significantly the range of his interests, he began to be interested in physical culture for pilots, their work standards, nutrition and deleterious factors related to their occupation, he demonstrated these factors in the work of engineering personnel, etc. Interest in these matters can be attributed to the participation of N. M. Dobrotvorskiy in a long-distance flight in the summer of 1927 over the route of Moscow—Borisoglebsk—Kharkov—Kiev—Moscow. This flight was made solely for the purpose of a comprehensive study of the work of the pilot and aircraft observer, and throughout the flight N. M. Dobrotvorskiy performed many physiological tests on himself and the crew. This was history's first long-term flight, not only in Soviet but worldwide aviation medicine, in which a flight surgeon participated for the purpose of in-depth investigation of pilot work under actual flying conditions. That same year, the title of RKKA Aircraft Observer was bestowed upon N. M. Dobrotvorskiy, i.e., he became the first pilot-physician in the history of Soviet aviation. He logged 56 h and 42 min between April 1925 and March 1928 aboard P-I, FS-IV, Yu-21 and ANT-3 aircraft.

One of the first investigations of N. M. Dobrotvorskiy was work on professiographic description of pilot work and psychophysiological analysis of professiograms. In a paper entitled "Zadachi psikhofiziologii letnogo truda" [Purposes of Psychophysiology of Flight Work] (1924),[2] he outlined a program for such description and anlysis which, in particular, stipulated that it was necessary "... 1) to become thoroughly acquainted with the process of flying and training; 2) to investigate the influence of a flight on the pilot as a function of its duration, altitude, rate of climb and descent ... not only during actual flight, but after it....; 3) establish the energy expenditure required of the pilot ... investigate his nutrition....; 4) determine at what point and under what conditions the pilot's job becomes harmful....; 5) determine the distinctions with respect to organization of the nervous system to be a pilot that are required to become a pilot....; 6) use appropriate tests to select the people needed for flying work." In an article entitled "Some of the Results of Work on Psychophysiology of Flying Work," which he published in the journal, VESTNIK VOZDUSHNOGO FLOTA, No 2, he reported that "the laboratory staff has already broken down all of a pilot's work into elements, defined the time for each operation, calculated reaction time of the visual, auditory and musculo-articular analyzers."

[2]"K istorii otechestvennoy aviatsionnoy psikhologii (dokumenty i materialy)" [History of Soviet Aviation Psychology (Documents and Material)], compiled by K. K. Platonov, V. V. Bobrova and S. Ya. Serova, Moscow, Nauka, 1981, p 317.

Unfortunately, no similar work was done thereafter with regard to systematic professiographic description of flying work as applied to new types of aircraft, which lowered appreciably the effectiveness of work on validation of requirements for the flying profession, improvement of the training system, etc.

At the first stage of operation of the Central Laboratory, N. M. Dobrotvorskiy and his colleagues conducted studies of pilot fatigability and, in particular, established its dependence on flight duration and number of landings.

As chief of the Central Laboratory, N. M. Dobrotvorskiy devoted much attention to supervision of scientific and practical work of specialists in psychophysiological laboratories of military schools of the Air Force and physicians of aviation units [chasti]. He prepared programs for this work, defined methodological procedures for investigations and analyzed their results. In 1926, he organized a mass-scale study of pilots by physicians in order to investigate flight work loads. The work asssignment provided for weighing flight personnel, measuring the chest and performing spirometry, taking cephalograms before and after a flight, keeping a log of daily flight work at the end of each week for one month. In spite of the limitation of methodological procedures, this work should be rated quite positively as an important means of training aviation physicians, for them to learn about the psychophysiological distinctions of flight work, inculcate interest and skill in independent research.

In spite of the definite achievements and enormous energy of N. M. Dobrotvorskiy in the matter of studying flight work, in 1928, as reported by A. S. Sergeyev,[3] he was forced to leave the Central Laboratory. The official reason for his departure was that he was discharged from the RKKA for health reasons.

Being unable to break with aviation, N. M. Dobrotvorskiy first found a job as non-T/O instructor of aviation medicine at the Air Force Academy imeni N. Ye. Zhukovskiy, where he was listed as instructor, aircraft observer first class in the aircraft laboratory, and from 1930 on he became a T/O instructor of aviation medicine at the same academy. During these years, he published two works on hygiene referable to flight clothing and organization of flying work.

The extensive knowledge he gained in the area of aviation as a result of participation in many flights, profound theoretical training and good knowledge of the basic aviation medicine problems enabled N. M. Dobrotvorskiy to produce the first Soviet manual of aviation medicine, entitled "Letnyy trud" [Flight Work] (1930). This book consisted of a course of lectures that the author delivered at the Air Force Academy imeni N. Ye. Zhukovskiy.

The typical distinction of this book is that it contains only the author's own views on different questions of aviation medicine, the results of his own research; he did not cover the achievements of aviation medicine in the USSR and abroad. In spite of this, the book has an abundance of new facts, new views and theoretical theses referable to many key issues of aviation medicine. The book reflects the great individual creativity of the author in diverse branches of aviation medicine.

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[3]A. A. Sergeyev, "Ocherki po istorii aviatsionnoy meditsiny" [Essays on the History of Medicine], Moscow —Leningrad, USSR Academy of Sciences, 1962.

It should be noted that anthropometric and biomechanical characteristics of a pilot, a method of calculating dimensional parameters, specifications for arrangement of the seat, pedals, controls, instrument panel, etc., were described in this book for the first time. N. M. Dobrotvorskiy was the first to warn about the adverse consequences of increasing the number of instruments in an aircraft cockpit, although at the time it did not exceed 15–18. He considered it mandatory to adapt the cockpit and its equipment to the physical capacities of a pilot. N. M. Dobrotvorskiy was actually the first to formulate the concept of the role of the human factor in assuring efficient and accident-free operation: "We believe that requirements can be imposed on man only after the aircraft is adapted to the requirements imposed on it by the average man."[4]

The book deals with questions of effects of noise, exhaust gases, low barometric pressure and hypoxia. The effect of accelerations is discussed the most comprehensively. The author was the first to cite data on G forces associated with linear and radial accelerations, he mentioned the possibility of blood shifts under the effect of centrifugal force, increase in weight of internal organs and possibility of their displacement, and in essence he disclosed the mechanism of action of accelerations.

N. M. Dobrotvorskiy made a thorough analysis of psychophysiological distinctions of work processes in the fighter pilot, reconnaissance pilot, bombardier and aircraft observer; he actually described the bases of professiographic investigation of performance and was the first to discuss question of pilots' work and rest schedules.

In 1935, N. M. Dobrotvorskiy began to work in the newly organized Institute of Aviation Medicine. In 2 years, we wrote several works dealing with arrangement and equipment of pilot work places, hygiene of aircraft cockpits, distinctions of aviation gunsights, ground-based bombardier training, control of accelerations, etc. In February 1936, his scientific achievements were acknowledged by conferring upon him the scientific degree of candidate of medical sciences and the scientific title of senior scientific associate. However, already in September 1937 he was transferred to the reserve and left his job at this institute.

His love for aviation, profound and extensive knowledge in the field of aviation medicine kept up his hope that he could still be useful to his beloved cause, to young aviation physicians. For this reason, in the very first days of the Great Patriotic War, N. M. Dobrotvorskiy volunteered on the front and served as chief of the medical service in several air force unit ["soyedineniya"]. Worsening of his health forced N. M. Dobrotvorskiy to retire at the end of the war. His illness progressed, and N. M. Dobrotvorskiy passed away in 1947.

The life and creative path traveled by one of the first Soviet flight surgeons, N. M. Dobrotvorskiy, are extremely interesting and instructive. As noted by A. A. Sergeyev, this path is interesting primarily because of the evolution that the views of N. M. Dobrotvorskiy underwent in his many years of solid association with aviation. He actively proclaimed that there was a need to restructure the guidelines

[4]N. M. Dobrotvorskiy, "Letnyy trud (kurs lektsiy, prochitannykh na kursakh usovershenstvovaniya nachsostava VVS pri Voyenno-vozdushnoy akademii im. N. Ye. Zhukovskogo)" [Flight Work (Course of Lectures Delivered in Classes for Advanced Training of Administrative Personnel of the Air Force at the Air Force Academy imeni N. Ye. Zhukovskiy)], Moscow, Air Force Academy, 1930.

for pilot screening and training on the basis of the Pavlovian teaching on higher nervous activity. N. M. Dobrotvorskiy actually proceeded along the trail blazed by experimental psychology and, being unable to reconcile in himself these two directions, he began to work on hygienic problems. The life of N. M. Dobrotvorskiy is also interesting because he reflected the distinctions of inception and development of our aviation and of Soviet aviation medicine which, having started with psychotechnics, gradually turned to development of the basic problems of physiological, hygienic and medical support of aviatoin specialists.

N. M. Dobrotvorskiy was the founder of the active apporoach to investigtion of psychophysiological distinctions of flying work, he was a pioneer in evaluation, from the standpoint of engineering psychology, the work place and, in particular, lay-out of instruments, validation of dimensions and forms of controls. He was the first to provide a physiological and hygienic description of flight personnel working conditions, he validated the principles and forms of interaction between the flight surgeon and flight personnel, etc.

The scientific legacy of N. M. Dobrotvorskiy enables us to comprehend the significance of early stages of development, patterns of emergence of Soviet aviation medicine, to appreciate highly the breadth and depth of his knowledge, scientific foresight, enormous need to devote all his energy and knowledge to development of aviation, improvement of working conditions of pilots and strengthening their health. Unquestionably, the work of N. M. Dobrotvorskiy, his scientific and organizational activities had an appreciable impact on subsequent development of Soviet medicine, and we can see in its advances the results of the work of this remarkable Soviet aviation physician.

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[Text] **Editorials**

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